Abstracts

O-001

A Polymorphism in a Let-7 microRNA Binding Site of KRAS in Women with Endometriosis. Olga Grechukhina,[1] Rafaella Petracco,[1] Shota Popkhadze,[1] Efi Massasa,[1] Trupti Paranjape,[2] Elcie Chan,[2] Idhaliz Flores,[3] Joanne Weidhaas,[2] Hugh Taylor.[1,4] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine; [2]Therapeutic Radiology, Yale University School of Medicine; [3]Microbiology; Obstetrics and Gynecology, Ponce School of Medicine and Health Sciences; [4]Molecular, Cellular and Developmental Biology, Yale University School of Medicine.*

Endometriosis is found in 5-15% of women of reproductive age and is more frequent in relatives of women with the disease. Activation of KRAS results in de novo endometriosis in mice, however activating KRAS mutations have not been identified in women. We screened 150 women with endometriosis for a polymorphism in a let-7 microRNA binding site (LCS6) in the 3'UTR of KRAS. We detected the KRAS-variant allele in 31% of women with endometriosis as opposed to 5% of a large diverse world population. Subjects with non-variant KRAS allele more commonly presented with pain (77% vs. 42%, p=0.0001) and dysmenorrhea (96% vs. 42%, p=0.0001) than those with the variant allele. Subjects with KRAS-variant more often suffered from infertility (40 % vs. 18%, p=0.003). Q-RT-PCR showed 3 times higher KRAS mRNA levels in endometrial stromal cells (ESC) from subjects with endometriosis positive for alternative allele compared to ESC from women with endometriosis carrying wild type KRAS allele (p=0.0049) and 9 times higher than in ESC from women without the disease (p=0.0001). Similar differences in KRAS protein expression were confirmed by WB. Increased KRAS protein expression was due to altered microRNA binding as demonstrated using luciferase reporter assays. Luciferase activity of a reporter carrying an alternative allele decreased by 70% when a specific siRNA targeting the mutant let-7 binding site on KRAS 3'UTR was used (p=0.049). ESC from women with the KRAS-variant showed 73% increase in proliferation rate (p=0.04) and 1.5 fold increase in their invasion capacity (p=0.013). In a murine model, endometrial xenografts containing the KRAS-variant demonstrated increased proliferation and decreased progesterone receptor expression. These findings suggest that an inherited polymorphism of a let-7 microRNA binding site in KRAS leads to abnormal endometrial growth and endometriosis. The LCS6 polymorphism is the first described genetic marker of endometriosis risk. The KRAS pathway presents a potential therapeutic target for treatment of endometriosis.

O-002

Relationship between Cervical-Vaginal Fluid Elafin Concentrations and Subsequent Cervical Shortening in Women at High Risk of Spontaneous Preterm Birth. Danielle Abbott, Evonne Chin-Smith, Seed Paul, Chandiramani Manju, Andrew Shennan, Tribe Rachel. *Division of Women's Health, Women's Health Academic Centre, KHP, King's College London, London, United Kingdom.*

Introduction: Elafin (SKALP), a natural antimicrobial peptide with antibacterial/antiprotease properties, is an important component of the innate immune system. It is hypothesised that elafin production in cervico-vaginal fluid (CVF) will be altered in women at risk of spontaneous preterm birth (STPB) associated with inflammation/infection.

Aim: The aim of this study was to determine the relationship between CVF elafin concentrations and cervical length in a cohort of woman at high risk of SPTB.

Methods: Elafin concentrations were measured in 437 CVF samples (taken at two-weekly intervals between 14-28 weeks') from 74 pregnant women recruited as part of a prospective longitudinal study of inflammation and cervical length (CLIC study). All women were asymptomatic but high risk for SPTB; controls (n=38, who did not develop a short cervix) and women who developed a short cervix <25mm (n=36) who were randomised to either cerclage or vaginal progesterone (400 mg/od). Elafin was measured by ELISA. Logged data were analysed using random-effects interval regression (Stata), with 28 low censored sample values and 85 high values.

Results: Mean concentrations of elafin were consistently higher in the CVF of the short cervix group, regardless of gestation and treatment (ratio 2.71, CI 1.94 to 3.79, p<0.0005). Elafin concentrations >200,000 pg/ml predicted cervical shortening from 14 weeks (n=11, ROC area = 1.00, p=0.0082), remained high when cervical shortening was first detected (ratio 3.03 CI 1.92-4.81, p<0.0005) and was consistently raised across gestation. Elafin concentrations were unaltered by treatment with cerclage or progesterone (ratio 1.28, CI: 0.88-1.87, p=0.196). Raised elafin concentrations before 24 weeks' were associated with SPTB <37 weeks (ratio 1.79, CI: 1.05-3.05, p=0.034).

Conclusion: This novel prospective study of high-risk asymptomatic women suggests that CVF elafin concentrations may be useful for the early prediction of SPTB prior to cervical shortening. Raised elafin concentrations may reflect a reactive response to the presence of infection rather than being a causative factor. We plan to evaluate the utility of elafin to predict SPTB and target therapies in a larger cohort.

Funding: Action Medical Research, Tommy's charity (1060508) WOW (239281)

O-003

Oogonial Stem Cells Isolated from Adult Human Ovaries Generate Oocytes In Vitro and In Vivo. Yvonne AR White,[1] Dori C Woods,[1] Yasushi Takai,[2] Osamu Ishihara,[2] Hiroyuki Seki,[2] Jonathan L Tilly.[1] *[1]Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA; [2]Department of Obstetrics and Gynecology, Saitama Medical Center/Saitama Medical School, Saitama, Japan.*

Introduction: Since the early 1950s, clinical management of problems associated with ovarian insufficiency and failure has been restricted by the belief that the pool of oocytes set forth at birth is not amenable to replacement or renewal. However, recent studies from several labs support that adult female mice possess rare oogonial stem cells (OSCs) that routinely produce new oocytes (Nature 2004 428:145; Nat Cell Biol 2009 11:631; Differentiation 2010 79:159). Extending these findings to humans would represent a paradigm shift in reproductive medicine by providing new avenues for management of ovarian function.

Objective: To isolate human OSCs (hOSCs) and assess their capacity for oocyte and follicle formation in vitro and in vivo.

Materials and Methods: As previously reported in mice (Nat Cell Biol 2009 11:631), immunological detection of cell-surface expression of VASA can be used to isolate OSCs. In the present study, viable hOSCs were isolated from ovarian cortex of 6 patients (22-33 years of age), under institutionally approved protocols following written informed consent, by combining cell surface expression of VASA with fluorescence activated cell sorting (FACS). Once established in culture, hOSCs were stably transduced with a GFP expression vector (GFP-hOSCs). For in vitro follicle formation, human ovarian cortical biopsies from 22-33 year old patients were dissociated and reaggregated with GFP-hOSCs. For in vivo follicle formation, GFP-hOSCs were directly injected into human ovarian cortical tissue biopsies (n=20), xenografted into NOD/SCID female mice, and collected 7-14 days later for assessment of GFP expression by immunohistochemistry.

Results: Following 72 hours of in vitro culture, ovarian aggregates contained large (50 microns) GFP positive cells tightly surrounded by ovarian somatic cells, in structures that closely resembled follicles. Ovarian xenografts contained easily discernible primordial and primary follicles with centrally-located GFP-positive oocytes.

Conclusions: The existence of hOSCs in women and their ability to generate oocytes and follicles in vitro and in vivo may offer new opportunities to expand and enhance current fertility preservation strategies.

Support: NIH R37-AG012279

O-004

Maternal Melatonin Protects Fetal Brain in Chronically Hypoxic Pregnancy. CM Cross, JA Hansell, EJ Camm, DA Giussani. *Physiology, Development and Neuroscience, University of Cambridge, United Kingdom.*

Intrauterine growth restriction (IUGR) is associated with an increased prevalence of oxidative stress injury in the developing brain (Padilla-Gomes et al. *Acta Paed.* **96(11)**:1582, 2007). A common cause of IUGR is placental insufficiency, decreasing oxygen and nutrient delivery to the fetus. The antioxidant melatonin is neuroprotective to the fetal brain in an ovine model of placental insufficiency (Miller et al. *Dev Neurosci*. **27**:200, 2005). However, the partial contributions of fetal hypoxia or undernutrition to triggering fetal brain oxidative stress in complicated pregnancy, and whether melatonin has neuro-protective effects in pregnancies selectively complicated by either challenge remains unknown. We compared the neuro-protective effects of maternal treatment with melatonin in fetal offspring of hypoxic or undernourished pregnancy in rats.

Methods: On day 15 of gestation, 42 Wistar rats were divided between 6 groups of n=7: control (C, 21% O2), hypoxic (H, 10% O2) or undernourished (U, 40% reduction in food intake) pregnancy, each +/- melatonin (5 microg/ml in maternal drinking water). At day 20 of gestation, brains were frozen for Western blot analysis from 1 male fetus per litter.

Results: Hypoxic or undernourished pregnancy promoted significant IUGR (fetal body weight, C =3.73±0.05, H= 2.94±0.03, U= 3.05±0.04, P<0.05) and a similar increase in fetal brain : body ratio (C =4.6±0.1, H= 5.9±0.1, U= 5.3±0.1, P<0.05). Hypoxic but not undernourished pregnancy significantly increased the fetal brain protein expression of heat shock (HSP) 70, nitrotyrosine (NT) and 4-hydroxynonenal (HNE) - three established molecular fingerprints of oxidative stress (Fig. 1). Maternal treatment with melatonin prevented the induction of fetal brain oxidative stress in hypoxic pregnancy.
Conclusion: Fetal brain oxidative stress damage in pregnancy complicated by placental insufficiency may be due to fetal hypoxia rather than fetal undernutrition. Maternal treatment with melatonin offers a plausible target for clinical intervention against the adverse effects of complicated pregnancy on neural development.
British Heart Foundation and the BBSRC.

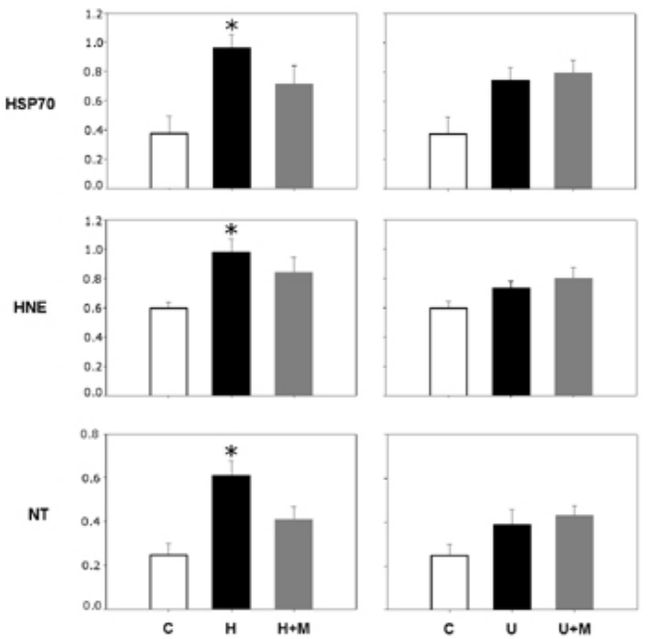

Fig. 1. Effects of control (C), hypoxic (H) or undernourished (U) pregnancy with or without melatonin (M) on the relative expression of HSP70, NT and 4-HNE in the fetal brain at the end of gestation. *P <0.05 compared to control (One-way ANOVA, with the Tukey's post hoc test.)

O-005

Decreased Smooth Muscle Expression Following Short Hairpin Mediated Knockdown of Hoxa11 in Murine Uterosacral Ligaments. Alexandra M McPencow, Marsha K Guess, Yan Ma, Alex M Hennessey, Kathleen A Connell. *Section of Urogynecology & Reconstructive Pelvic Surgery, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: Pelvic organ prolapse (POP) is a common, debilitating disorder in women. The uterosacral ligaments (USLs) provide apical support to the vagina, and have been shown to be attenuated in women with POP. Hoxa11 is a conserved homeobox gene involved in the differentiation of the genital tract. Previously we showed that Hoxa11 is necessary for the development of the USLs in mice and that there is decreased expression of HOXA11 along with decreased cellularity in the smooth muscle (SM) in the USLs of women with POP. Alterations in SM protein expressions have been implicated in the development of POP.
Objective: The aim of this study was to determine if a causal relationship exists between Hoxa11 expression and SM proteins, including α-SMA, transgelin, desmin and calponin. We also evaluated the expression of caldesmon, which inhibits SM contraction and has been reportedly increased in USLs in women with POP.
Methods: Twelve week old C57/BL6 mice were injected with 16µg of either empty vector (control (CTL), n=8) or Hoxa11 shRNA plasmid (knockdown (KD), n=9) into the uterus, cervix and peritoneal cavity. USLs were harvested 48 hours after transfection. Messenger RNA levels of Hoxa11, α-SMA, transgelin, desmin, calponin and caldesmon were measured via real time-PCR using tubulin as an internal control. The 2(-DeltaDelta C(T)) method was used for data analysis.
Results: Hoxa11 mRNA levels were significantly decreased in the USLs of KD vs. CTL mice (P=0.004). The expression of α-SMA mRNA was decreased 2.1 fold (P=0.04), transgelin decreased 1.6 fold (P=0.04), desmin decreased 1.9 fold (P=0.00) and calponin decreased 2 fold (P=0.02) in KD vs. CTL mice USLs. In contrast, caldesmon mRNA expression showed significant upregulation in KD USLs by 1.6 fold (P=0.04).
Conclusion: Hoxa11 KD in murine USLs leads to decreased expression of smooth muscle contractile apparatus genes and increased expression of a gene regulating inhibition of SM contraction. Thus, HOXA11 mediated pathways may be involved in not only the myogenesis of USLs, but also in maintenance in the inherent property of USLs. Further studies on upstream regulation of the genes involved in SM apparatus and contraction in USLs will provide a greater understanding of the pathogenesis of POP.

O-006

Dehydroepiandrosterone Slow-Releasing Intrauterine Pellets Inhibit Implantation. Kenan Omurtag, Patricia Jimenez, Antonia I Frolova, Daniel Cusmano, Kelle Moley. *Obstetrics and Gynecology, Washington University-St Louis, St Louis, MO, USA.*
Background
Endometrial stromal cells (ESC) undergo hormone-driven decidualization as a requirement for embryo implantation. Recently, we demonstrated that 6-aminonicotinamide (6-AN) and dehydroepiandrosterone (DHEA) inhibit the pentose phosphate pathway (PPP) leading to decreased decidual marker expression in vitro and decidualization in vivo. Additionally, we have shown that mice fed a DHEA diet experience decreased ovulatory rates and poor oocyte quality. These results suggest that PPP inhibition by either compound may be a suitable target for use in an intrauterine contraceptive device (IUD). Here we present pregnancy outcomes in mice receiving a placebo or DHEA pellet in the uterine horn.
Methods
ICR female mice had a placebo (n=18) or DHEA (1.5mg/60 days)(n=20) pellet placed into one uterine horn after index delivery. Females were housed with males of proven fertility for 120 days. Number of pups/litter and serum DHEA concentrations were quantified. The pellets were custom-formulated (Innovative Research, Inc). All studies were approved by the Animal Studies Committee and followed NIH guidelines for Animal Care and Use.
Results
The average pups/litter was reduced by 55% in the first litter after placement of the pellet in the DHEA group (13.0 vs. 5.9 pups/litter, p=0.002). Furthermore, the DHEA group had fewer pups/litter during the 60-day active phase of the pellet compared to the placebo (11.3 vs 8.2, p<0.001). After 60 days, both groups had a similar number of pups (12.6 vs. 10.1, p=0.1). Additionally mean serum concentrations of DHEA were slightly elevated, but within normal range for our laboratory (0.512 vs. 0.265 ng/mL, p=0.07) in the DHEA vs. control group, respectively.
Conclusion:
By disrupting glucose utilization via the PPP, DHEA and 6-AN prevent decidualization of the ESC both in vitro and in vivo. We have also shown that DHEA disrupts ovulation through this same pathway. Here, we demonstrate that intrauterine pellets containing DHEA impair embryo implantation leading to smaller litters. Moreover, this effect is reversible as litter size returns following the 60-day timed-release of the pellets. Our findings suggest DHEA, a hormonal inhibitor of the PPP, released slowly in an intrauterine pellet, may have a contraceptive effect acting locally on the endometrium. Investigation of these and other non-hormonal inhibitors of the PPP, such as 6-AN, warrant further study as novel IUD.

O-007

A Nine-Herb Formulation, Suppresses Growth of Endometriotic Tissue Implants in a Mouse Model of Endometriosis. Jillian M Liu,[1] Angel A Rivera,[1] Jasmine R Bryant,[1] Michael Walden,[1] Winston Thomas,[2] Robert N Taylor,[1] Neil Sidell,[1] Friedrich Wieser.[1] *[1]Gynecology and Obstetrics, Emory University School of Medicine; [2]Obstetrics and Gynecology, Morehouse School of Medicine.*
Objective:
Channel Flow, a nine-herb formulation (HM-09), was shown to reduce inflammatory and angiogenic responses in primary endometrial stromal cells. We hypothesized that this formulation might alter endometriotic antigenicity

or immunoregulatory factors that affect specific functions of peritoneal macrophages, and hence inhibit invasion and proliferation of endometriotic tissue. In this study, we investigated the effects of HM-09 on the establishment and growth of endometriotic tissue implants in a mouse model of endometriosis.
Methods:
We have established conditions to reproducibly transplant green fluorescent protein (GFP)-expressing endometrial tissue from donor mice into the peritoneal cavity of syngeneic recipient mice. Three days before inoculating the endometrial tissue, the recipient mice were gavaged with either HM-09 treatment (prepared as a tea) or water (control group). After 17 days of treatment mice were scarified. Implant number and size were evaluated 14 days post endometrial tissue injection. Peritoneal fluid washings were collected at necropsy and assayed for the presence of vascular endothelial growth factor (VEGF) with a mouse ELISA kit (RayBiotech, Norcross, GA). Data are presented as mean ± SD. Mann-Whitney U test was performed and a p-value of < 0.05 was considered statistically significant.
Results:
15 mice were treated with HM-09 and 19 mice with vehicle. HM-09-treated mice showed a reduced number of implants (3.0 ± 2.2) compared to the vehicle treated mice (5.2 ± 2.5), ($p < 0.05$). Also, endometriotic lesions in HM-09-treated mice had a smaller mean volume (11.4 ± 13.9 mm^3) when compared to control mice (28.9 ± 24.0 mm^3), ($p < 0.05$). Finally, ELISA showed a 50% decrease in peritoneal fluid VEGF levels of the HM-09 treated group (97.5 ± 61.1 pg/ml) compared to the controls (195.6 ± 84.3 pg/ml, $p < 0.05$).
Conclusions:
Our results indicate that HM-09 administered as a tea can reduce the establishment and slow the development of endometriotic implants in a murine model. We postulate that HM-09 inhibits growth of endometriotic lesions by suppression of angiogenic growth factor genes. In future studies, we will explore the mechanisms by which HM-09 prevents endometriotic lesion formation in vivo.
NIH-Support: U01 HD66439; 1R21HD065115-01; R01HD55379.

O-008

Decreased Expression of miR-451 Alters YWHAZ (14.3.3ζ) in the Eutopic (EUE) and Ectopic Endometrium (EcE) in a Baboon Model of Endometriosis. Niraj Joshi,[1] Renwei Su,[1] Bruce Lessey,[2] Asgerally Fazleabas.[1]
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MiR's which regulate gene expression have been postulated to play a role in endometriosis. To identify changes in miR's in the EUE and EcE of baboons following the induction of endometriosis, and validate their potential target genes, total EUE RNA extracted from the same baboons (n=3) before and 3 m after the induction of endometriosis were analyzed on a 8x15K miR microarray (Agilent). The signal data were preprocessed by AgiMiRna and an empirical Bayes model was used to estimate the p-values. The potential mRNA targets were predicated using TargetScan 5.1. This study focused on the analysis of miR-451 and its 3 targets CDKN2D, GATAD2B, and YWHAZ. YWHAZ, which regulates cell proliferation and apoptosis, was further characterized by IHC and 3' UTR luciferase assays. Induction of endometriosis results in significant changes of several miRs (miR 451, 181a, 200a, 19b, 424, 21, 29c and 141), of which miR-451 was the most highly down regulated ($p< 0.000036$) in the midsecretory phase compared to controls. QRT-PCR analysis for miR-451 revealed a continuous decrease in expression at 1, 6, 9, and 15 m in both the EUE and EcE. However, pri-miR-451 expression was significantly increased suggesting an altered biogenesis of miR-451 processing. The temporal decrease in miR-451 expression leads to significant increases in the expression of its targets CDKN2D, GATAD2B and YWHAZ. YWHAZ protein was also significantly increased in the glandular epithelium of EUE. In vitro 3' UTR luciferase assay resulted in a significant reduction in YWHAZ in the cells transfected with the miR-451 expression vector compared to control. Comparative studies from women with endometriosis also showed a significant reduction in miR-451 during the midsecretory phase. This study suggests that an altered cell proliferation and apoptotic response is associated with an increased expression of YWHAZ mediated by miR-451 in endometriosis. The in vitro transcriptional anlysis confirmed the in vivo findings. In conclusion our studies reveal a crucial role for miR-451 in regulating the expression of genes involved in cell proliferation, nucleosome remodeling, and apoptosis which are physiological responses associated with the pathophysiology of endometriosis in both baboons and women. (**U54-HD40093**)

O-009

The Efficacy of Nitric Oxide Donor on Cervical Ripening Prior to Operative Hysteroscopy: A Randomized, Double Blinded, Controlled Trial. Chonticha Chencheewachat, Srithean Lertvikool, Matchuporn Sukprasert, Boonsri Chanrachakul. *Obstetrics and Gynecology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*
Objective: To evaluate the efficacy of the intravaginal isosorbide mononitrate (IMN) on cervical ripening before operative hysteroscopy in nonpregnant women.
Methods: This study was conducted as a double-blind, randomized, placebo-control trial and was approved by the Ethical Committee.Written informed consent was obtained from each participant. Women who were scheduled for operative hysteroscope were recruited for this study. Subjects were randomly assigned to receive either 40 mg of IMN (n = 29) or placebo (n= 24) vaginally for four hours before underwent operative hysteroscopy. Cervical dilatation before operative hysteroscopy was assessed using Hegar's dilators starting from number 1, followed by subsequent larger size until it found resistance through the internal os. The size of dilator that passed through the internal os before resistance was found was recorded as the cervical width. Details of operation and complications were noted.
Results: Mean cervical width in IMN group was significantly wider than in placebo group (6.8 (1.6) mm vs 5.0 (2.1) mm, $p <0.01$) . Adverse effects and operative complications were not significantly different between the two groups. There was no serious complication in both groups.
Conclusion: Isosorbide mononitrate was more effective than placebo for cervical ripening before operative hysteroscopy.

O-010

Relationship between Peritoneal Endometriotic Lesion Innervation and Pain Perception in Women with Endometriosis. Kathleen M Peters, Sri P Maharajaa, Ian S Fraser. *Department of Obstetrics, Gynaecology and Neonatology, University of Sydney, Sydney, NSW, Australia.*
Introduction
Endometriosis is a common, benign gynaecological condition frequently associated with severe pelvic pain. Pain perception and severity of disease do not correlate. Likewise, pain mechanisms are poorly understood. Our group has previously demonstrated increased nerve fibre density (NFD) and nerve growth factor (NGF) expression in the peritoneal lesions of endometriosis illuminating possible aspects of pain mechanisms in those with chronic pelvic pain.
Objective
For the first time we aimed to study the relationship between pain perception and quantifiable physiological findings (NFD and NGF levels) in peritoneal lesions in a group of women with endometriosis.
Methods
Peritoneal lesions were stained using established immunohistochemical procedures. Sympathetic, parasympathetic and sensory NFD and NGF expression were assessed in the glands, stroma, and adjacent sub-peritoneal tissue of peritoneal lesions. Pain scores were calculated using a Visual Analogue Pain Scale identifying perception of pain during menstruation, inter-menstrual pain and pain during coitus.
Results
Sensory and sympathetic, but not parasympathetic, NFD were significantly higher in the lesion core (glandular epithelium and stroma) compared with adjacent peritoneum (both $p<0.001$). Significantly higher NGF expression was noted in the glandular epithelium as compared to lesion stroma and adjacent peritoneum ($p=0.026$). Increasing sympathetic NFD correlated with increasing severity of menstrual pain ($p=0.04$). No other correlations between NFD and pain scores were observed. There was also no correlation between increased NGF expression in peritoneal lesions and pain scores.
Discussion
The unique findings of this study indicate that pain mechanisms in endometriosis are more intricate than simply peritoneal lesion innervation. Activation of pelvic nociceptive pathways by various noxious stimuli may result in CNS sensitization; suggesting a neuropathic component to endometriotic pain. Elucidating complex pain mechanisms may lead to mechanism-targeted therapeutics to alleviate this debilitating symptom of endometriosis.

O-011

Gene Variants Associated with Early Menarche Are Also a Risk Factor for Endometriosis. Kenneth Ward, Rakesh Chettier, Pam Farrington, Hans Albertsen. *Research, Juneau Biosciences, Salt Lake City, UT, USA.*

Early menarche is consistently observed to be a clinical risk factor for endometriosis, while late menarche is associated with a decreased risk of subsequent endometriosis. Recently, variants in or near several genes have been associated with age at menarche (Elks et al. Nature Genetics, Vol. 42, 1077-1085, 2010). The findings include genes associated with body mass index (FTO, SEC16B, TRA2B and TMEM18), genes implicated in energy homeostasis (BSX, CRTC1 and MCHR2) and genes implicated in hormonal regulation (INHBA, PCSK2 and RXRG). We hypothesized that polymorphisms in these genes could also influence a woman's risk of developing endometriosis.
Methods: The study is a hypothesis-based subset analysis of our ongoing genome-wide study of genes contributing to endometriosis. To minimize confounding factors we included only case and control individuals of Northern-European ancestry. 2168 surgically confirmed endometriosis patients and 1998 population controls met inclusion criteria. We examined single nucleotide polymorphisms linked to candidate loci previously associated with age of menarche. DNA extraction and genotyping was performed by standard methods.
Results: We found that many of the early menarche polymorphisms also show association with endometriosis. For some markers we only saw association when the patient had early menarche, but for most loci the endometriosis association was present regardless of the patient's age of menarche. Sample data are shown in the Table below.

rs number	Associated Gene	MAF n=1900 Controls	MAF n=299 Early Menarche Endometriosis	p-Trend	Odds Ratio
Published Early Menarche Genes					
rs2947411	TMEM18	0.17	0.20	0.000826	1.28
rs2687729	EEFSEC	0.26	0.29	0.006881	1.17
rs17171808	KDM3B	0.18	0.15	0.001273	0.79
New Candidates Based on Endometriosis Patients with Early Menarche					
rs7819743	PRDM14	0.03	0.08	7.1E-09	2.74
rs9637198	COL18A1	0.25	0.34	7.9E-07	1.58
rs6074396	SPTLC3	0.28	0.36	1.2E-05	1.50

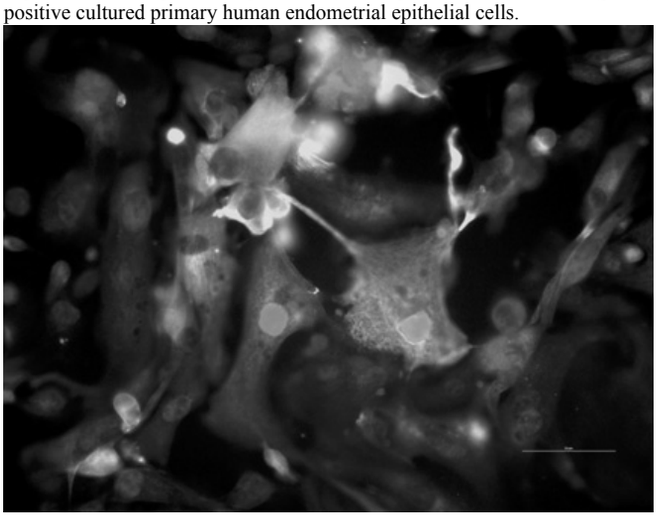
Conclusion: These data suggest that early menarche genes are also associated with endometriosis. Early menarche associated gene variants may be modifying loci with respect to endometriosis or they may reflect common biologic pathways underlying both phenotypes. It will be important to test whether these genetic pathways contribute to other conditions associated with early menarche.

O-012

Combined Bioinformatics and Immunological Approach Identified FOXD3 in Human Endometrium; Implications for Endometriosis. N Tempest, U Sajjad, J Drury, A Valentijn, N Wells, O Vasieva, DK Hapangama. *University of Liverpool.*

Introduction: Endometriosis is a common gynaecological disease with unknown pathogenesis. We employed Bioinformatics tools to systematically collate available information, identifying the key players in the pathogenesis of endometriosis. Immuno-histochemistry, immunofluorescence(IF) and western blotting were used to confirm the presence of one of the identified transcription factors.
Methods: Bioinformatics tools were used to identify the interconnectivity and interlinking of the expression of genes, proteins and cellular functional aberrations associated with endometriosis. Most important transcription factors were identified and we hypothesised that women with endometriosis will have different expression profiles of those transcription factors to fertile controls. Twenty endometrial biopsies from women with (n=10) and without (n=10) endometriosis were analysed for the expression of FOXD3.
Results: A group of transcription factors including FOXD3, which are likely to play a key role in regulating aberrant cellular functions and genes that are described in endometriosis were identified. FOXD3 immuno-staining was observed in endometrial stromal and epithelial compartments in both groups of women. In the fertile control women there was significantly decreased FOXD3 in the Window of implantation when compared with proliferative phase(p=0.03). However, this pattern was reversed in women with endometriosis(p=0.008). In the proliferative phase endometrial FOXD3 staining was significantly decreased in women with endometriosis(p=0.008).
Conclusion: To our knowledge FOXD3 has not been previously described in the human endometrium. It is a transcription factor, which affects the pluri-potency factors Oct 3/4 and Nanog, both of which have been described in the endometrium. It may be important in determining the cell fate in endometrial stem cell. We propose that Bioinformatics is a valuable tool in directing basic and translational research.

Figure: An IF image showing FOXD3(red) expression in cytokeratin(green) positive cultured primary human endometrial epithelial cells.



O-013

Deep-Sequencing Based Detection and Characterization of Placental Microrna from Maternal Circulation: A Potential Biomarker for Fetal Well-Being. Zev Williams,[1,2] Iddo Z Ben-Dov,[2] Aleksandra Mihailovic,[2] Rony Elias,[3] Sebastian Frey,[2] Zev Rosenwaks,[3] Thomas Tuschl.[2] *[1]Obstetrics and Gynecology, Albert Einstein College of Medicine, New York, NY, USA; [2]Tuschl Laboratory, The Rockefeller University, New York, NY, USA; [3]CRMI, Weill-Cornell Medical Center, New York, NY, USA.*

Objective
MicroRNAs (miRNAs) are short (~22nt) non-coding RNAs that regulate gene expression and play an essential role in development, differentiation and disease. Unlike conventional methods for miRNA quantitation such as qRT-PCR and microarrays, miRNA cDNA library sequencing allows detection of novel miRNAs and identification of point mutations and processing variability and quantitation. Levels of some miRNAs within the placenta are altered in several pregnancy-related disease conditions including preeclampsia. The purpose of this study was to determine whether miRNAs originating from the placenta could be directly cloned and sequenced from maternal plasma thereby allowing for non-invasive profiling of placental-specific miRNAs as a potential biomarker.
Methods
Total RNA was extracted from placental and 18 adult tissues, sequentially ligated using bar-coded adapter oligonucleotides, reverse transcribed and PCR amplified. The resulting cDNA was then pooled and the multiplexed library was deep-sequenced using Illumina Hi-Seq technology. To identify miRNA with a placental-specific expression pattern we examined between-tissue differential expression of miRNA using heatmap representation of hierarchically clustered tissues and miRNA precursor clusters. Peripheral blood samples from 4 trios (mother, father and umbilical cord blood) and non-pregnant women and corresponding placental tissues were similarly processed.
Results
Ten miRNA clusters were identified as placental-specific/enriched, most notably members of the mir-498 cluster on chromosome 19 (104-fold expression above median). Analysis of differential expression revealed that mir-143(2) (20-fold), mir-127(8) (36-fold), mir-134(41) (20-fold), mir-498(46) (38-fold) and mir-204(1) (16-fold) cluster members were specific to mothers and umbilical cord plasma compared to controls. Placental-derived miRNA contained both unique sequence and length variability.
Conclusion
Placenta-specific miRNAs can be directly cloned and sequenced from maternal venous plasma thereby allowing non-invasive monitoring of placental miRNAs and the potential use as a non-invasive biomarker for placental disease.

O-014

Functional Genomic Analysis of Twin-Twin Transfusion Syndrome Recipient Amniotic Fluid Reveals Major Alterations in Fetal Brain Gene Expression and Up-Regulation of Apoptosis. Lisa Hui,[1] Heather C Wick,[2] Kenneth J Moise, Jr,[3] Anthony Johnson,[3] Kirby L Johnson,[1] Diana W Bianchi.[1] *[1]Mother Infant Research Institute, Tufts Medical Center, Boston, MA, USA; [2]Department of Computer Science, Tufts University, Medford, MA, USA; [3]Texas Center for Fetal Treatment, University of Texas, Houston, TX, USA.*

Background: We have previously shown in euploid fetuses that amniotic fluid supernatant (AFS) contains RNA from multiple fetal organs, including brain.
Aim: To understand the molecular mechanisms underlying the recipient's response to twin-twin transfusion syndrome (TTTS) and identify fetal biomarkers by performing functional genomic analysis of AFS.
Methods: This was a prospective study analyzing cell-free RNA transcripts in recipient AFS from women undergoing clinically-indicated laser surgery for TTTS. Control AFS samples matched for gestational age and fetal sex were obtained from singleton fetuses undergoing genetic amniocentesis. AFS from 6 recipient twins (4 Quintero Stage II and 2 Stage III, GA 17 - 22 w) and 6 controls (GA 16 - 21 w) were included. We used the dependent *t* test with Benjamini-Hochberg correction to evaluate up-regulated genes in TTTS vs euploid controls. Functional analyses of up-regulated genes were performed with Ingenuity™ software.
Results: Paired analysis of TTTS vs control AFS identified 961 genes significantly up-regulated in TTTS cases. Among the most statistically significant biological processes that differed between cases and controls were skeletal and muscular disorders, neurological diseases and inflammatory disease. There were 70 nervous system genes associated with the functional annotation of tauopathy in TTTS cases: 6 of these are specifically expressed by fetal brain (*DCX, TUBB, TUBB3, NRXN1, DNM3, CAMK2B*). Water transporter genes (*AQP6* and *AQP12A/12B*) and vascular endothelial growth factor receptor 1 (*FLT-1*) were also up-regulated in TTTS cases vs controls. The most enriched cellular/molecular functions in TTTS cases were cell death (apoptosis), cellular assembly and organization, and cell signaling.
Conclusions: Our study provides novel molecular evidence of the impact of TTTS on multiple fetal organs including the nervous system. Apoptosis is a significant biological pathway up-regulated in recipient twins. The fetal brain-specific genes up-regulated in TTTS cases represent potential biomarkers of neurodevelopmental outcome.

O-015

Maternal Diabetes Leads to Rat Fetal Cardiac Hyperplasia and Dysfunction in an Experimental Rat Model. Lara Lehtoranta,[1] Mervi Haapsamo,[2] Olli Vuolteenaho,[2] Juha Rasanen.[3] *[1]Obstetrics and Gynecology, University of Turku, Turku, Finland; [2]Physiology, University of Oulu, Oulu, Finland; [3]Obstetrics and Gynecology, University of Eastern Finland, Kuopio, Finland.*

OBJECTIVE To investigate whether pre-gestational diabetic milieu would lead to fetal cardiac cellular, genetic, and functional alterations.
STUDY DESIGN Ten diabetic rat dams with 107 fetuses (control dams = 20, fetuses = 219) were preconceptually injected with STZ (35 mg/kg). Fetal ultrasonography in isoflurane anesthesia was performed on GD 13—14, 16—17, and 19—21 (Acuson Sequoia 512, Mountain View, California). After the last examination the dam was killed and fetal hearts were gathered. TUNEL assays were used to detect apoptosis (diab = 23, control = 39). HE- samples (diab = 46, control = 85) were used for morphometric analysis (apoptosis, mitosis, erythroblasts). Gömöri silver samples (diab = 21, control = 22) were used for cardiomyocyte dimension analysis. Fetal heart RNA (diabetic = 15, control = 14) was isolated (Qiagen Rneasey reagents, Netherlands) and hybridized with cDNA probes. Expression was measured with qRT-PCR using TaqMan chemistry on a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA). Newborn pups from separate litters were used for heart and body weight analysis (diab = 10, control =9).
RESULTS Diabetic fetal hearts had elevated counts of apoptosis, mitosis, and erythroblast-to-erythrocyte-ratios. Cardiac-to-thoracic-area-ratio, heart weight and heart-to-body-weight-ratio were all increased in the diabetic fetuses. Cell-size and amounts of cell nucleus per fixed area were similar in diabetic and control group. At GD 13—14 holosystolic atrio-ventricular-valve-regurgitation was present, outflow tract mean velocity was decreased and inflow E-to-A-wave-ratio increased in diabetics. Throughout, diabetic fetuses had decreased heart rates. Increased levels of ANP and BNP, and skeletal heavy myosin isoforms (MYH2, MYH3) mRNAs were recorded. Genetic level changes were seen in genes linked to action potential (KCNIP2), hypoxia (EGLN3), and metabolism (UCP3, GLUT4).
CONCLUSION Maternal hyperglycemia leads to fetal myocardial enlargement caused by hyperplastic growth. Fetal cardiac dysfunction at GD 13—14 was present in holosystolic AVVR and decreased cardiac output. These changes were transient. We present differences of expression in metabolic, cardiac growth, and functional process genes. Furthermore, there was evidence of hypoxic insult in the diabetic fetuses.

O-016

Resveratrol Prevents Maternal Hypertension and Improves Fetal Growth in ENOS Knock-Out Mice. Rajan Poudel,[1] Christian F Reuda-Clausen,[1] Joanna L Stanley,[1,2] Colin P Sibley,[1,2] Sandra T Davidge,[1] Philip N Baker.[1,2] *[1]Departments of Obstetrics/Gynecology and Physiology, University of Alberta, Edmonton, Canada; [2]Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom.*

Background
Preeclampsia (PE) and fetal growth restriction (FGR) complicate up to 10% of all human pregnancies and contribute significantly to fetal and maternal morbidity and mortality. Resveratrol, a polyphenol found in a number of plants, has been shown to improve many pathological features associated with PE and FGR (1). We have previously reported that endothelial nitric oxide synthase knock out mice (eNOS-/-) exhibit hypertension during pregnancy and deliver growth-restricted pups in comparison to wild type (C57Bl6/J) mice (2). We hypothesized that Resveratrol supplementation during pregnancy in eNOS-/- mice would decrease blood pressure (BP) and increase fetal weight.
Methods
Pregnant eNOS-/-mouse were randomly assigned to receive either Resveratrol supplemented diet (4 g/kg diet; n=5) or control diet (n=5) between day (d) 0.5 and 18.5 of gestation. BP was measured using the tail-cuff method on d10.5 and d17.5. Dams were sacrificed on d18.5 and uteri examined for number of live fetuses and resorptions. Fetuses and placentas were blotted dry, weighed and examined for gross abnormalities. All data are presented as mean±SEM and were compared using a two sample unpaired t-test.
Results
Resveratrol supplementation increased fetal weight (Control diet: 0.90 ± 0.01g, Resveratrol suppl: 0.98 ± 0.01g, p= 0.008), such that fetal weights from Resveratrol supplemented eNOS-/- mice approximated to those of wild type (C57Bl6/J) mice (1.07 ± 0.01g n=6). Blood pressure on d10.5 was not different (Control diet: 126±7 mmHg, Resveratrol suppl: 132±6 mmHg, p=0.5) between the groups. Resveratrol, however, reduced BP on d17.5 of gestation in these mice (Control diet: 142±7 mmHg, Resveratrol suppl: 115±5 mmHg, p=0.01). Again, this approximated to that of wild type mice on d17.5 (122 ± 5 mmHg, n=9). There were no effects of Resveratrol on resorptions, placental weight or fetal weight/placental weight ratio. No evidence of gross abnormalities was observed in fetuses from Resveratrol supplemented dams.
Conclusion
Resveratrol ameliorated hypertension and fetal growth restriction in the eNOS-/- model, and has potential as a therapeutic strategy.
References
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2. Stanley et al. Repro Sci 2011.

O-017

Quantitative Label-Based Uterine Endothelial Protein Profile for Chronic Binge-Like Alcohol Exposure Exposure. Jayanth Ramadoss,[1] Ronald R Magness.[2] *[1]Ob/Gyn, UTMB, Galveston, TX, USA; [2]Ob/Gyn, UW, Madison, WI, USA.*

Introduction: A cardinal feature of Fetal Alcohol Spectrum Disorders (FASD) is Intrauterine Growth Restriction (IUGR). IUGR models show dysfunctional uterine vascular adaptations. Although alcohol has pregnancy-specific effects on both the maternal systemic & uterine endothelium, large scale quantitative proteomic studies are lacking. **Objective**: We utilized ITRAQ-label based method & nanoLC MS/MS to quantitate alcohol-induced alterations in the maternal uterine endothelial proteome. **Methods**: Uterine artery endothelial cells from pregnant ewes (Day 120–130; term=147), were FAC sorted, validated, & cultured. To mimic maternal binging patterns, 4 pairs of cell lines derived from 4 pregnant ewes were treated for 3 h on 3 days in a compensating system without or with alcohol (300 mg/dl) for 2 weeks. Lysates were TCA precipitated, solubilized, trypsin digested before addition of isobaric ITRAQ tags. Following SCX fractionation, samples were analyzed by nano LC MS/MS (MS Bioworks). Data were searched using MASCOT, quantitated & analyzed by Student's t test using Scaffold Q+. **Results**: Cell viability was unaltered by alcohol. Independent of treatment, a total of 363 proteins were detected

with ≥2 unique peptides/protein & 0% false discovery rate (FDR). Of the 31 proteins significantly (P<0.05) altered by alcohol, 14 were upregulated (↑) & 17 downregulated (↓). These proteins include those related to cell structure (eg. ↓tubulin β, P=0.002; ↓vimentin, P<0.001), transcription & translation regulation (eg. ↓elongation factor 1α, P<0.001; ↑40S ribosomal protein S19, P=0.002; ↓calreticulin, P=0.004), histones (eg. ↑H4, P<0.001; ↑H2B-1k, P=0.036), Ca^{+2}/nitric oxide (NO) (eg. ↓HSP90β, P=0.014; ↑calmodulin, P<0.001), & redox balance (eg. ↓thioredoxin, P=0.017). In addition to 100% FDR in these analyses, we also performed gel based 2-D Dige analysis. **Conclusions**: 1) This is the first study to utilize the well established ITRAQ-labeled technology for proteomic analyses on any cell type in FASD field; 2) Chronic binge alcohol has specific effects on the maternal uterine vasculature at the level of the endothelial proteome; 3) The data support binge alcohol effects at multiple levels including epigenetic, transcriptional & translational; 4) Alcohol has major effects on proteins related to redox balance & Ca^{+2}/NO regulation; & 5) These findings support a role for the uterine compartment in FASD pathogensis. NIH HL49210, HL83144, AA19446, HD38843

O-018

Local Administration of Ad.VEGF-A_{165} to the Utero-Placental Circulation Enhances Fetal Growth and Reduces Brain Sparing in an FGR Model of Guinea Pig Pregnancy. Vedanta Mehta,[1] Michael Boyd,[4] Hannah Barker,[4] Adnan Avdic-Belltheus,[4] David Carr,[1] John Martin,[2,3] Ian Zachary,[2] Donald Peebles,[1] Anna L David.[1] *[1]Institute for Women's Health, UCL, London, United Kingdom; [2]Rayne Building, UCL, London, United Kingdom; [3]Ark Therapeutics Ltd, , London, United Kingdom; [4]BSU, Royal Veterinary College, London, United Kingdom.*

Introduction: Fetal growth restriction (FGR) is commonly caused by impaired utero-placental perfusion limiting fetal nutrient and oxygen supply. We have shown that adenovirus (Ad) mediated over-expression of VEGF-A_{165} in the uterine arteries (UtAs) of mid-gestation pregnant sheep significantly increases UtA blood flow (~37%) for at least 30 days post-injection, compared with UtAs transduced with a control adenovirus encoding β-galactosidase (Ad.LacZ). This was concomitant with a significant reduction in vascular contractility and significant adventitial neovascularization.

Aim: To study if Ad.VEGF-A_{165} transduction enhances fetal growth in the FGR guinea pig.

Methods: To create FGR, virgin Dunkin-Hartley guinea pigs were nutrient restricted peri-conceptually. Under general anaesthesia at mid-gestation (30-34 days of gestation), sonographic fetal measurements were recorded in 9 nutrient restricted pregnant sows and compared with data from fetuses of control *ad lib* fed sows. At laparotomy the UtAs were dissected free of fat, and the UtAs and radial arteries on each side were transduced externally with $5x10^9$ viral particles of Ad.VEGF-A_{165} or Ad.LacZ, using a thermosensitive pluronic gel. The guinea pigs were sacrificed 31-34 days post-surgery but before birth. Fetal organ weights and biometry were recorded.

Results: There was no maternal or fetal morbidity and mortality. Nutrient restriction resulted in a 40% reduction in fetal weight and brain sparing. Administration of Ad.VEGF-A_{165} led to an increase in fetal weight (94.5±2.01g, n=11) compared to control Ad.LacZ treated fetuses (84.9±2.81g, n=10, p=0.061). The liver and kidney weights were significantly higher in the Ad.VEGF-A_{165} group (5.6±0.23g v/s 4.7±0.18g, p=0.019 and 0.74±0.065g v/s 0.37±0.021g, p<0.001 respectively), and the brain/liver weight ratio was significantly lower (0.45 ± 0.019 v/s 0.53 ± 0.017, p=0.021), suggesting an attenuated brain sparing effect.

Conclusion: Ad.VEGF-A_{165} transduction of the utero-placental vasculature enhances fetal growth and reduces brain sparing in nutrient restricted fetal guinea pigs, and may be of potential benefit in ameliorating FGR.

O-019

Prenatal Ad.VEGF Gene Therapy Increases Fetal Growth Velocity and Expression of VEGF Receptors in an Ovine Paradigm of Fetal Growth Restriction. David J Carr,[1,2] Raymond P Aitken,[2] John S Milne,[2] Donald M Peebles,[1] John F Martin,[3,4] Ian C Zachary,[3] Jacqueline M Wallace,[2] Anna L David.[1] *[1]Maternal & Fetal Medicine, UCL Institute for Women's Health, London, United Kingdom; [2]Rowett Institute of Nutrition & Health, University of Aberdeen, Aberdeen, United Kingdom; [3]Centre for Cardiovascular Medicine & Biology, UCL, London, United Kingdom; [4]CSO, Ark Therapeutics, London, United Kingdom.*

Introduction: Adenovirus (Ad) mediated over-expression of vascular endothelial growth factor (VEGF) in the uterine arteries (UA) increases uterine blood flow (UBF) in normal sheep pregnancy. Herein effects of Ad.VEGF on UBF, growth velocity and body/organ weights of growth-restricted sheep fetuses and placental VEGF/receptor expression in late gestation were investigated in the overnourished adolescent paradigm.

Methods: Singleton pregnancies were established by embryo transfer in 57 adolescent ewes subsequently overnourished to restrict placental/fetal growth or fed a control diet. At 89±1.5d gestation, overnourished ewes were randomised to receive $1x10^{12}$ particles Ad.VEGF (n=18), Ad.LacZ (n=14) or saline (n=13) injected into each UA at laparotomy. Controls received saline (n=12). Transonic flowprobes were fitted around UAs supplying the gravid horn. Fetal biometry/wellbeing was assessed blind by weekly ultrasound (single operator). UBF was monitored on alternate days until necropsy at 131±1.6d. Placental mRNA expression of VEGF and its receptors (Flt1/KDR) was determined in maternal caruncle and fetal cotyledon by quantitative real time RT-PCR.

Results: There was no significant effect of Ad.VEGF on UBF. Fetal abdominal circumference (AC) was greater in Ad.VEGF vs. Ad.LacZ/saline groups at 112±0.1d and 119±0.1d gestation (p=<0.001-0.047). Necropsy weight was not significantly different between overnourished groups but fewer fetuses weighed more than 2SD below the control mean in Ad.VEGF vs. Ad.LacZ+saline groups (5/18 vs. 18/27, p=0.038). Serial ultrasound biparietal diameter:AC ratios, relative brain weight and brain to liver weight ratios were lower (p=<0.001-0.046) and caruncular Flt1 and KDR mRNA expression was higher (p=0.028/0.034) in Ad.VEGF vs. Ad.LacZ+saline groups.

Conclusion: Ad.VEGF treatment significantly increased fetal growth velocity at 0.77–0.82 gestation with evidence of an attenuated "brain sparing" effect and increased Flt1/KDR mRNA expression in the maternal placental compartment. These effects occurred in the absence of any measurable change in UBF.

O-020

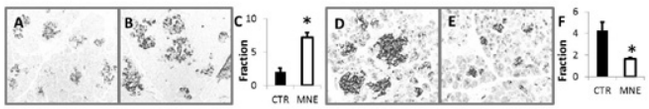
Maternal Obesity (MO) and Maternal Nutrient Excess (MNE) in Baboon Pregnancy Stimulate Pancreatic Islet β-Cell Insulin (INS) Content but Decrease IGF-II: Potential Role of Amino Acids (AA). Peter W Nathanielsz, Mark J Nijland, Thomas J McDonald, Cun Li. *OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

INTRODUCTION: MO increases OFF predisposition to T2D and obesity. We have developed an MO/MNE model in baboon pregnancy to evaluate fetal pancreatic development at 0.9 gestation (G).

HYPOTHESIS: MO/MNE increases fetal pancreatic INS and IGF-II accompanied by reduced nutrient availability.

METHODS: Female baboons ate chow (CTR - 12% energy fat; 0.29% glucose, 0.32% fructose; n=7) or MNE - 45% energy fat; 4.62% glucose and 5.64% fructose plus unlimited fructose sodas for at least nine months before pregnancy; N=5) that increased body fat (DEXA) and triglyderides (TG). Fetuses removed by CSection at 0.9 G under general anesthesia and maternal and fetal blood measured for Gl, INS, APO-B and C-peptide and immunohistochemistry (IHC) for INS and IGF-II. Fetal plasma amino acids (AA) measured by HPLC. Analysis - Student's ttest; * P set at < 0.05

RESULTS: Plasma (CTR first, MNE second): Maternal: GL - 53.4 ± 7.9 vs. 62.8 ± 14.3 $mg.dl^{-1}$; INS 31.3 ± 21.4 vs. 40.5 ± 22.6 $uU.ml^{-1}$; C-peptide 1.4 ± 0.62 vs. 1.72 ± 0.62 $mg.dl^{-1}$. Fetal GL 55.7 ± 4.8 vs. 59.0 ± 8.8; $mg.dl^{-1}$; INS 5.3 ± 1.3 vs. 6.2 ± 1.6 $uU.ml^{-1}$; C-peptide 0.98 ± 0.21 vs. 2.16 ± 0.84 $mg.dl^{-1}$. Fetal TG and cholesterol were not different but fetal APO-B was higher in MNR than CTR (p< 0.07) and fetal C-peptide difference approached significance (p<0.07). MO fetal pancreas IHC showed more islet INS and less IGF-II vs. CTR (Fig. 1). AA play an important role in pancreatic development both as secretagogues and growth factors. Fetal taurine (Fig 1), serine, glycine, and methionine (not shown) important one-carbon cycle AA were all reduced (p< 0.05) Fig 1.



Fetal pancreatic IHC for INS A) CTR fetus, B) MNE fetus, C) pooled data for fraction stained; IGF-II D) CTR fetus, E) MNE fetus, F) pooled data for fraction– M ± SEM; * $p < 0.05$.

CONCLUSIONS: MO accelerates fetal pancreatic INS production but decreases IGF-II an essential growth factor and AA key to pancreatic development.

O-021

Cathepsin B Is Required for the Clearance of Dying Ovarian Follicles. Janelle Luk,[1] Rajinder Dawra,[2] Ashok Saluja,[2] Joshua Johnson.[1] *[1]Department of OB/GYN & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Tumor Biology and Progression Research Program, University of Minnesota Medical School, Minneapolis, MN, USA.*

We have shown that inhibition of the nutrition-responsive mammalian Target of Rapamycin (mTOR) *in vivo* results in oocyte loss and female sterility in the fruit fly *Drosophila melanogaster.*

Similarly, treatment of mouse and human ovarian follicles *in vitro* with the mTOR inhibitor Rapamycin (RAP) results in the development of 'empty' follicles lacking oocytes. We term this novel mode of oocyte loss follicle regression. In this process, granulosa cells take on a phagocytic phenotype to clear neighboring dying granulosa cells and to destroy the enclosed oocyte. In a *Drosophila* screen, we identified the protease Cathepsin B (CTSB) as being required for follicle regression during RAP feeding. We hypothesized that this role for CTSB would be conserved in mammals and assessed the ovarian phenotype of the CTSB knockout mouse (CTSBKO). CTSBKO mice were found to exhibit apoptotic granulosa cell bodies in all follicles, even those growing follicles that also contained mitotic granulosa cells. This phenotype is thus distinct from the classical view of atresia where only non-growing follicles contain apoptotic bodies. Further, all knockout corpora lutea exhibited high numbers of apoptotic luteal cells. Last, highly degenerate follicles were found in knockout ovaries that contained metaphase II eggs, suggesting that follicle regression and oocyte clearance were compromised. Interestingly, CTSBKO mice also exhibited premature ovarian failure, where mice failed to produce litters past 5 months of age, at which point their ovarian reserve of primordial follicles was exhausted. CTSB is thus implicated in both follicle regression and in the maintainence of the normal duration of ovarian function.

O-022

FSH Induction of LHR in Murine Granulosa Cells Is Dependent, in Part, on the Protein Kinase A Regulatory-Rho-Guanine Nucleotide Exchange Factor, AKAP13 (a.k.a. Brx). Kate Devine, Marcy Maguire, Xiuye Xing, Paul Driggers, X Catherine Guo, Alan DeCherney, James H Segars. *Reproductive Endocrinology & Infertility, Eunice Kennedy Shriver National Institutes of Child Health and Human Development, National Institutes of Health.*

Objectives: The protein A kinase (PKA) anchoring protein 13 (AKAP13) is highly expressed in the ovarian follicle and is known to regulate PKA activation through binding of the regulatory subunit. We previously reported that AKAP13 expression was induced during follicular development in mice and humans. Since follicle stimulating hormone (FSH) leads to activation of PKA, we sought to determine the role of AKAP13 in FSH receptor signaling in granulosa cells.

Methods: Ovaries were harvested from C57Bl/6 mice, and granulosa cells (GCs) were isolated and cultured. RT-PCR was used to compare transcripts of AKAP13, LHR, and aromatase after 12, 24, and 48hrs' treatment with recombinant FSH at 1IU/mL. To determine whether baseline and FSH-induced LHR expression was AKAP13 dependent, the same experiments were performed with the addition of 24 hrs' treatment using either AKAP13 or negative control siRNA. Finally, since LHR expression is critical for ovulation, AKAP13 +/+ males mated with +/+ females vs. females haploinsufficient for AKAP13 (+/-), and litter sizes were compared.

Results: FSH treatment significantly induced LHR (p=0.03), and aromatase (p=0.02), and showed a trend towards induction of AKAP13 (p=0.13) transcripts. Control experiments confirmed that AKAP13 siRNA transfection reduced AKAP13 expression 5-fold (p=0.005). AKAP13 knockdown lowered basal transcripts of aromatase (p=0.02) but had no effect on basal expression of LHR. AKAP13 siRNA transfection reduced FSH induction of LHR. Among 60 total matings, litter sizes from +/- females averaged 0.7 pups fewer than those of +/+ females, although the differences were not significant.

Conclusions: In addition to its known induction of LHR, FSH induced AKAP13 in murine GCs. Furthermore, knockdown of AKAP13 reduced FSH induction of LHR. Our findings suggest that AKAP13 may be required for optimal induction of LHR during folliculogenesis.

O-023

Obese Women Exhibit a Pro-Inflammatory Follicular Environment during Ovarian Hyperstimulation That May Impact Reproductive Outcome. Ronit Haimov-Kochman,[1] Ido Eldar,[1] Dana Finci-Yeheskel,[2] Francine Lossos,[1] Arye Hurwitz,[1] M Levin,[2] Simcha Urieli-Shoval.[2] *[1]IVF Unit, Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [2]Hematology Unit, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*

Background Obese women experience longer times to conception, even if they are young and cycling regularly, which is suggestive of alterations in ovarian function during the periconceptual period.

Objective This study sought to determine whether there are alterations in the preovulatory follicular environment that are likely to influence oocyte developmental competence in obese patients.

Design, Setting, and Participants Women attending a University affiliated infertility clinic were categorized into body mass index (BMI) groups of normal (n = 23; BMI 18–24.9 kg/m2), overweight (n = 21; BMI 25–29.9 kg/m2), and obese (n =18; BMI >30 kg/m2).

Intervention For each patient, follicular fluid was recovered from single follicles at oocyte retrieval, granulosa cells were pooled from multiple follicular aspirates and cumulus cells were pooled after separation from the oocytes.

Main Outcome Measures Follicle fluid was assayed for C reactive protein (CRP) and Serum Amyloid A (SAA). Granulosa and cumulus cells were analyzed for mRNA expression of SAA.

Results Increasing BMI was associated with increased follicular fluid CRP (r=0.39, p=0.002) and SAA (r=0.37, p=0.007). Follicular fluid SAA level was positively correlated with CRP level in the follicle (r=0.6, p<0.0001). The concentration of CRP and SAA in the follicle was correlated with their plasma levels (r=0.94, p<0.0001; (r=0.92, p<0.0001, respectively). Pregnancy rates were 17.7% for the obese group, which was about half the rate observed in the normal and overweight groups.

Conclusions Obese women exhibit an altered pro inflammatory ovarian follicular environment, which may be associated with poorer reproductive outcomes typically observed in these patients.

O-024

Steroid Hormone Regulation of Ovarian Germline Stem Cell Differentiation. Chonthicha Satirapod, Ning Wang, Yvonne AR White, Dori C Woods, Jonathan L Tilly. *Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.*

Background: Adult mouse ovaries possess germline or oogonial stem cells (OSCs) that generate oocytes in vitro and fertilization competent eggs in vivo [Nature 2004 428:145; Nat Cell Biol 2009 11:631; Differentiation 2010 79:159]. In unpublished studies using suicide gene technology, disruption of OSC differentiation into oocytes in young adult mice results in reversible oogenic failure. Other work has shown that Stra8, a germ cell-specific meiotic commitment gene, is expressed in mouse ovaries during the estrogen (E2)-dominating follicular phase but is lost during the progesterone (P4)-dominating luteal phase. That E2 and P4 cooperate to regulate OSC differentiation may explain how the primordial follicle pool in mice remains relatively stable during the first 3 months of life despite large numbers of oocytes being lost through atresia [Nature 2004 428:145; Reproduction 2006 132:95].

Objective: To examine if steroids coordinate OSC differentiation into oocytes in adult ovaries.

Methods: For in-vivo studies, age-matched young adult female mice were treated with E2, P4 and E2 plus P4; ovarian tissues were collected 24 hours later for analysis of Stra8 expression and follicle numbers. For in-vitro studies, OSCs isolated from adult mouse ovaries were cultured with E2, P4 or E2 plus P4 for 24 hours, and evaluated for Stra8 activation. Chromatin immunoprecipitation (ChIP) was used to examine if activation of estrogen receptors (ER) triggered occupancy on estrogen response elements in the Stra8 promoter.

Results: E2 treatment increased ovarian Stra8 expression and primordial follicle numbers, consistent with elevated numbers of primordial follicles being detected during metestrus (versus other stages of the cycle) following the endogenous surge in E2 [Am J Anat 1923 31:439; Cell 2005 122:303]. While P4 alone had no effect, the ability of E2 to increase Stra8 expression and oocyte numbers in adult ovaries was abolished by P4. Likewise, E2 induced

Stra8 expression in OSCs in vitro; while P4 alone had no effect, it abolished E2-induced Stra8 activation. Gene expression analysis showed that ER-alpha, but not ER-beta, is expressed in OSCs, and ChIP confirmed that E2-activated ER associates with the Stra8 promoter.
Conclusion: Ovarian steroids orchestrate differentiation of OSCs into oocytes in adult mouse ovaries via transcriptional regulation of Stra8.
Support: NIH R37-AG012279

O-025

Disruption of *Chtf18* Causes Impaired Fertility and Defects in Male Meiosis. Karen Berkowitz,[1] Fang Yang,[2] Peijing Wang,[2] Lydia Koenig,[1] Dawnette Urcuyo,[1] Fahmida Khan,[1] Aislinn Sowash,[1] Thomas Jongens,[3] Klaus Kaestner.[3] *[1]OB/GYN and Biochemistry & Molecular Biology, Drexel University College of Medicine; [2]Developmental Biology, University of Pennsylvania School of Veterinary Medicine; [3]Genetics, University of Pennsylvania School of Medicine.*
INTRODUCTION: Factors that reduce germ cell number or interfere with gametogenesis can limit or even preclude reproduction, leading to infertility, which is a major medical concern affecting about 1 of every 10 individuals of childbearing age worldwide. Defects in chromosome segregation during meiosis can result in infertility, birth defects, and pregnancy loss, and the mechanisms underlying chromosome mis-segregation are poorly understood. *CTF18* (chromosome transmission fidelity factor 18) encodes an evolutionarily conserved subunit of the Replication Factor C-like complex that is necessary for accurate chromosome segregation in yeast, and is crucial for fertility in the fruit fly. Previously, we demonstrated that *Chtf18*, the orthologue of *CTF18* is expressed throughout the germline of the mouse, suggesting a role for *Chtf18* in mammalian gametogenesis (Berkowitz et al., 2008). Our goal is to elucidate the role of *CTF18*/*Chtf18* in mammalian germ cell development.
METHODS: We employed gene targeting to derive mice that lack a functional Chtf18 protein. The phenotypic consequences of *Chtf18* deletion were assessed by gross, histological, chromosomal, and immunofluorescence examination of *Chtf18*-deficient compared to wild-type gonads. Statistical analysis was performed using the Student t-test and ANOVA. All data were expressed as mean standard error of the mean (SEM), and p values less than 0.05 were considered statistically significant.
RESULTS: Adult *Chtf18*-deficient testes were much smaller and morphologically abnormal compared to those of their wild-type adult littermates. Seminiferous tubules of *Chtf18*-deficient testes demonstrated a significant deficiency of spermatogenic cells, and tubules contained large multinucleated and aberrant-appearing cells. Sperm concentrations were reduced more than 5- fold and fertility was significantly impaired in *Chtf18*-deficient males. Surface spread analysis of *Chtf18*-deficient spermatocyte nuclei revealed that homologous chromosomes separated prematurely during meiosis I, and that DNA double strand repair was defective.
CONCLUSION: *Chtf18* plays critical roles in male fertility and meiosis in mammals.

O-026

Glucose Transporters in Human Sperm. Erica Schoeller, Samantha Schon, Kelle Moley. *OBGYN, Washington University in St Louis, Saint Louis, MO, USA.*
Background: Sperm cells require large amounts of energy to support the hyperactivity and acrosome reaction necessary for fertilization of an oocyte. Sperm cells encounter a variety of environments en route from the testis to the oocyte and must adapt to utilize various substrates as energy sources. GLUTs are a family of 14 facilitative glucose transporters that transport a variety of substrates, including glucose and fructose, across cell membranes. These transporters have specific substrate affinities and subcellular localization, allowing cells to tightly regulate hexose entry and metabolism. Sperm cells contain a unique set of GLUTs and their tight regulation of hexose metabolism allows sperm cells to adapt to the different environments of the reproductive tracts and also to control metabolism during peak energy periods such as the hyperactivated motility prior to ooctye fertilization. The goal of this study was to identify and quantify the specific set of glucose transporters in human sperm cells to better understand how these cells meet their metabolic needs during the fertilization process.
Methods: RNA was extracted from Percoll-purified sperm cells and converted to cDNA. Quantitative real-time PCR was performed using primers specific for each GLUT. Primers sets were evaluated for efficiency and specificity and a standard curve was generated for each amplicon. Results were then converted to copy number per nanogram of RNA.
Results: GLUTs 1,3,4,5,8,9, and 12 were detected in human sperm cells by absolute qRT-PCR. GLUT8 is the most abundant transporter expressed in sperm cells, followed by GLUT3 and then GLUT5. The presence of these GLUTs was also confirmed by western blot. We next examined the cellular localization of GLUTs 1,4,8, and 12 by immunofluorescence. GLUT1 and GLUT12 localize to the acrosome of the sperm cell, GLUT8 localizes to the connecting piece of the sperm cell, and GLUT4 localizes specifically to the sperm tail.
Conclusions: Substrate utilization for sperm cells is a highly dynamic process, dependant on the local environment. Sperm cells express a variety of hexose transporters, and for the first time, the expression of insulin-responsive GLUTs 4 and 12 was detected in human sperm cells. This suggests that insulin signaling may be important in sperm cells, and possibly function to increase glucose uptake in response to insulin. This could potentially play an important role in the hyperactive motility of sperm required for fertilization.
Funding: R01 HD40390-09

O-027

Differential microRNA Expression in Human Spermatozoa from Smokers. Timothy H Marczylo,[1] Akwasi A Amoako,[1] Justin C Konje,[1] Timothy W Gant,[2] Emma L Marczylo.[2] *[1]Endocannabinoid Research Group, Reproductive Sciences Section, CSMM, University of Leicester, United Kingdom; [2]Systems Toxicology Group, MRC Toxicology Unit, Leicester, United Kingdom.*
Introduction: Smoking is associated with a decrease in sperm count and sperm motility. Recent work has suggested that environmental chemicals, including those contained in cigarette smoke can have adverse effects not just on the exposed individuals, but can be transmitted through the germ line to the next (unexposed) generation. The mechanism(s) by which smoking elicits these detrimental cross generational effects is unknown. MicroRNAs (miRNA) are non-coding, regulatory RNA implicated in the establishment of permanent heritable epigenetic alterations.
Aims: To establish that sperm contain miRNAs and that they are altered by smoking
Methods: Microarray analysis of miRNA was performed on RNA extracts from non-smoking (n=5) and smoking (n=5) men with normal semen parameters. Quantitative real-time PCR (qRT-PCR) was employed to confirm differential expression. Validated targets of differentially expressed miRNAs were downloaded from the miRWalk database and subjected to pathway analysis
Results: Human spermatozoa contain 800 individual miRNAs. 46 miRNA s were significantly ($p<0.05$) differentially expressed in specimens from smokers. Differential expression of 4 of these miRNAs (MiR340, MiR365, MiR129* and MiR634) was confirmed by qRT-PCR. Cell and tissue proliferation and differentiation appear to be the predominant networks mediated by these altered miRNAs.
Conclusions: Changes to the expression profile of miRNA in sperm provide a potential mechanism by which smoking-induced phenotypes could be transmitted through the male germ line. Processes mediated by miRNA in sperm are likely to be important in early-development. The top diseases and disorders associated with the observed miRNA changes are developmental and genetic disorders, including reproductive system disease.

O-028

Discovery and Characterization of piRNAs in the Human Ovary – An Evolutionarily Conserved Mechanism To Guard the Germline Genome. Zev Williams,[1,2] Pavel Morosov,[2] Aleksandra Mihailovic,[2] Pavan Pavankumar,[3] Thomas Tuschl,[2] Zev Rosenwaks.[3] *[1]Obstetrics and Gynecology/Human Genetics, The Albert Einstein College of Medicine, Bronx, NY, USA; [2]RNA Biology- Tuschl Laboratory, The Rockefeller University, New York, NY, USA; [3]Reproductive Endocrinology, Weill Cornell Medical Center, New York, NY, USA.*
Objective
piRNAs are a newly discovered class of small RNAs that guard the germ cell genome from genetic rearrangements and mutagenesis caused by transposons. Loss of piRNAs result in severe genetic damage and sterility. piRNAs are present in male and female germ cells of lower organisms such as flies and worms. However, in humans and other higher organism, piRNAs have been detected only in male germ cells. Their absence in female germ cells has raised the dilemma of how the genome of the mammalian female germline is protected. The objective of this study was to use insights from developmental biology and new molecular biology techniques including small RNA cloning and massively paralleled high-throughput sequencing to detect piRNA in the human female germline.

Design
IRB approved experimental study.
Materials and Methods
Since piRNA expression in the male germline is limited to developing spermatocytes, we focused on detecting piRNA in the fetal ovary, the period when germ cells are in a similar developmental state. Total RNA was extracted from human fetal ovaries and 19-35 nt long RNA fragments were sequenced using high-throughput next-generation sequencing. Fetal testis, somatic tissue and adult testis and ovaries were similarly assayed.
Results
147 piRNA clusters were identified in the human fetal ovary. Adults testis contained piRNA clusters while adult ovary and fetal somatic tissues did not. 15 clusters were conserved between testis and ovary and the remainder were unique to the ovary. Clusters were evenly distributed along both strands of all chromosomes and ranged in length from 1-11 kb.
Conclusions
This is the first report of piRNAs in the human ovary. The presence of piRNAs in the female germline of higher organisms provides an evolutionarily conserved molecular mechanism for protecting the germline genome from damage caused by transposons. Disruption of this pathway would be expected to result in infertility and provides a new target for investigation.
Support
HHMI and NIH (1K08HD068546-01)

O-029

microRNA-200a Serves a Key Role in the Decline of Progesterone Receptor (PR) Function Leading to Labor. Koriandr C Williams,[1] Nora E Renthal,[1] Jennifer C Condon,[2] Carole R Mendelson.[1] *[1]Depts of Biochemistry and Ob/Gyn, University of Texas Southwestern Med Ctr, Dallas, TX, USA; [2]Dept of Ob/Gyn, University of Pittsburgh, Pittsburgh, PA, USA.*
During most of pregnancy, uterine quiescence is maintained by increased PR activity, while spontaneous labor is facilitated by a concerted series of biochemical events that impair PR function. We postulate that near term PR function may be compromised within myometrium by increased local metabolism of P_4. Previously, we discovered that miR-200 family members, miR-200b and miR-429, and their targets, transcription factors ZEB1 and ZEB2, serve as P_4-modulated mediators of contraction-associated genes in pregnant uterus from mice to humans. In the present study, we identified a novel role for miR-200a in promoting increased metabolism of P_4 within mouse and human myometrium during the progression to labor. We observed that miR-200a exerts this function through direct repression of its target, signal transducer and activator of transcription, STAT5b, a known repressor of the P_4 metabolizing enzyme, 20α-hydroxysteroid dehydrogenase (20α-HSD). Here we report that miR-200a expression markedly increased and STAT5b mRNA and protein levels coordinately decreased in myometrium of pregnant mice as they progressed to labor and in myometrium of women in labor at term, as compared to that of non-laboring women. These changes were associated with dramatic increases in expression and activity of 20α-HSD in laboring myometrium from pregnant mice and women. Notably, knockdown of STAT5b in cultured human myometrial cells (hTERT-HM) caused an increase in 20α-HSD mRNA, whereas, overexpression of STAT5b decreased 20α-HSD expression. Moreover, overexpression of miR-200a in cultured hTERT-HM cells markedly suppressed STAT5b and increased 20α-HSD expression. Importantly, P_4 treatment of ovariectomized mice caused a decline in miR-200a expression, an increase in STAT5b mRNA and protein and decreased 20α-HSD mRNA in myometrium. By contrast, induction of preterm labor by treatment of 15 day pregnant mice with the PR antagonist, RU486, caused upregulation of miR-200a, suppression of STAT5b and induction of 20α-HSD in myometrium, compared to gestation-matched controls. Together, these findings implicate the miR-200 family as a crucial regulator of increased local P_4 metabolism in the pregnant uterus near term and in the decline in PR function leading to labor.
NIH-P01-HD011149; March of Dimes 21-FY11-30.

O-030

Choriodecidual Infection: A Molecular Basis for Preterm Premature Membrane Rupture. Jeroen Vanderhoeven,[1] Ryan McAdams,[3] Richard Beyer,[2] Theo Brammler,[2] Frederico Farin,[2] Michael Gravett,[1,4] Craig Rubens,[4] Kristina Adams-Waldorf.[1] *[1]Obstetrics & Gynecology, University of Washington; [2]Department of Environmental and Occupational Health Sciences, University of Washington; [3]Division of Neonatology, Seattle Children's; [4]Global Alliance to Prevent Prematurity and Stillbirth, Seattle Children's, Seattle, WA, USA.*
Background:
Early events leading to intrauterine infection remain poorly defined, but may hold the key to a therapeutic intervention for preterm birth. The study objective was to identify molecular pathways associated with an early choriodecidual infection for the first time in a well-controlled experimental model that replicates human pregnancy.
Methods:
Ten chronically catheterized pregnant monkeys (*Macaca nemestrina*) at 118-125 days gestation (term=172 days) received choriodecidual inoculation of either: 1) Group B Streptococcus 1 x 10^6 colony forming units (n=5) or 2) saline (n=5). Cesarean section was performed 4 days after GBS or 7 days after saline infusion. RNA was extracted from chorioamnion (inoculation site) and profiled using microarray. Statistical analysis focused on single genes and gene set analysis. Results were validated by RT-PCR (chorioamnion) and Luminex (amniotic fluid).
Results:
Significant elevations of cytokines in AF (TNF-α, IL-8, IL-1β, IL-6) were detected in GBS animals versus controls ($p<0.05$). Only two GBS animals developed early labor and chorioamnionitis with no cervical change in the remaining animals. Despite the lack of pathologic evidence of infection in three GBS animals, there were 603 genes differentially expressed in the GBS exposed chorioamnion versus controls ($p<0.05$; fold change >2.0). A striking downregulation of genes encoding cytoskeletal elements necessary for cellular tensile strength was observed in the single gene and gene set analysis (e.g. cytokeratin 6A, laminin, desmoplakin, desmoglein, keratinocyte differentiation). Upregulated genes identified many previously implicated (MMP1, IL-6, PECAM-1) as well as novel genes (e.g. placenta-specific 8, DCP2 decapping enzyme, myosin heavy chain 11).
Conclusion:
Our well-controlled nonhuman primate model enabled us to identify key pathways in the chorioamnion during early choriodecidual infection for the first time. We found induced inflammatory responses may not be apparent on histopathology, but may weaken the cytoskeletal support and epithelial integrity predisposing to preterm premature rupture of membranes.

O-031

Synthetic Progestins, Medroxyprogesterone Acetate (MPA), 17 α Hydroxyprogesterone Acetate (17OHPA) and 17 α Hydroxyprogesterone Caproate Inhibit Progesterone Metabolism in Human Cervical Fibroblasts. Jennifer J McIntosh, Eric J Kndutson, Kimberly E Hyatt, Dean A Myers. *Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.*
Background: Preterm birth remains a significant obstetrical problem and is a major cause of neonatal morbidity and mortality. In humans, cervical ripening is facilitated by a local progesterone withdrawal involving progesterone metabolism via 20α reductases (aldoketo reductase (AKR) 1C1, C2 and C3) and 5α reductase. 17OHPC has gained recent popularity for reducing the risk of preterm birth. However, the exact mechanism(s) via which 17OHPC act remains to be elucidated. Of interest, a closely related progestin, MPA, is a potent inhibitor of the AKR1C enzymes. MPA and 17OHPC differ only by the addition of a 6 methyl group in MPA and at the 17 position: caproate vs acetate.
Objective: Based on these similarities, we hypothesized that 17OHPC would inhibit progesterone metabolism in human cervical fibroblasts (HCF). We compared 17OHPC to MPA and 17OHPA.
Methods: HCF were seeded in 96 well poly-D Lys coated tissue culture plates at 40,000 cell/well. Metabolism of progesterone (1ng/ml) was determined at 0.5, 1, 2 and 3 h at 37C. The ability of MPA, 17-OHPA and 17-OHPC to inhibit progesterone metabolism was determined by using equal molar concentrations of synthetic progestin. Progesterone metabolism was determined using a Progesterone ELISA (cross reactivity: 5β -pregnan-3α -ol- 20-one, 6.7%; 17OHP, 17OHPA, 17OHPC, 0.5%; 5α/β -pregnan-3α ,20α -diol, <0.01%). Student's t-test with Bonferroni's correction was used to test for significance.
Results: 83.3+3.12% of progesterone was metabolized by 1 hour. 17-OHPC exerted a minimal, albeit significant ($p<0.05$) effect on progesterone metabolism (83.3+3.12% vs 63.4+7.19%), while 17-OHPA (83.3+3.12% vs 48.7%+9.47%)

and MPA (83.3+3.12% vs 34.7+9.35%) exhibited the greatest inhibition ($p<0.01$). As predicted, MPA exhibited an approximately 50% inhibition at a 1:1 molar ratio (58.3% inhibition). 17-OHPA was nearly as efficacious as MPA exerting a 42.6% inhibition while 17OHPC exerted only a 34% inhibition at a 1:1 molar ratio.

Conclusions: Synthetic progesterone supplementation may prevent preterm birth through local inhibition of progesterone metabolism in cervical fibroblasts. MPA and 17OHPA are the most effective inhibitors and this may have implications for progestin selection to prevent preterm birth.

O-032

Progesterone Regulates Synthesis of Cervical Extracellular Matrix from Primary Cervical Fibroblasts in Two-Dimensional and Three-Dimensional Culture. Michael D House,[1] Serkalem Tadesse,[2] Errol R Norwitz,[1] David L Kaplan.[3] *[1]Obstetrics and Gynecology, Tufts Medical Center, Boston, MA; [2]Obstetrics and Gynecology, Yale University; [3]Biomedical Engineering, Tufts University.*

Objective: Progesterone supplementation prevents preterm birth in women with a short cervix, but the mechanism is unclear. We hypothesize that progesterone acts by altering the composition of cervical extracellular matrix (ECM). We tested this hypothesis using primary human cervical fibroblasts in both two-dimensional (2D) and three-dimensional (3D) culture.

Methods: Cervical fibroblasts were isolated from cervical biopsies from non-pregnant women. Fibroblasts were culture expanded in DMEM containing 5% charcoal-stripped FBS and estradiol 10-8 M. For 2D culture, cells from passage 4-5 were seeded in six well plates and cultured for 4 weeks with / without progesterone (10-7 M, 10-6 M). Cells were assayed for progesterone receptors (PR-A, PR-B by western blot), metabolic activity (AlamarBlue), tissue wet weight, and collagen production (hydroxyproline). For 3D culture, cells were seeded on a previously validated, porous silk scaffold system and cultured for 6 weeks. The cells proliferated in the pores (500 um) and synthesized ECM. 3D cultures were tested for morphology and collagen extractability. For extractability experiments, collagen was extracted using 0.5 M acetic acid + pepsin (0.1mg/mL) at 4C for 48 h, and extracts run on SDS-PAGE, stained with Coomassie Blue, and compared to purified type 1 human collagen.

Results: PR-A and PR-B receptors were present. PR-B was enriched in the nuclear fraction whereas PR-A was enriched in the cytosolic fraction. Supplementation of 2D cultures with progesterone was associated with decreased metabolic activity, decreased tissue wet weight, and decreased collagen production ($p<.05$ for both doses). In 3D cultures, the absence of progesterone (but presence of estradiol) was associated with new ECM synthesis that uniformly covered the scaffold. Supplementation with progesterone resulted in non-uniform ECM synthesis and empty pores. The electrophoretic mobility of collagen chains extracted from 3D culture was similar to purified human collagen.

Conclusion:

Progesterone regulated production of ECM from primary cervical fibroblasts in 2D and 3D culture. Progesterone supplementation was associated with morphological differences in 3D culture, which could be useful in dissecting the effect of progesterone on the cervix in vivo.

O-033

HIF-1α-Induced Transcriptional Networks Orchestrate IL-8 Gene Expression in Cervical Stromal Cells. Annavarapu H Kishore, Ruth A Word. *Obstetrics and Gynecology, University of TX Southwestern Medical Center, Dallas, TX, USA.*

The mechanisms by which the cervix remains closed during uterine expansion of pregnancy are unknown. Previously, we showed that several cytokine genes (e.g., IL-8) are transcriptionally repressed by a cervix-specific isoform of the transcription factor MiTF (Micropthalmia associated Transcription Factor). Loss of MiTF during cervical ripening results in activation of these genes. Here, we sought to understand the regulatory mechanisms by which MiTF is suppressed during cervical ripening. **Methods:** Microarray analysis, qPCR, immunoblots, immunohistochemistry, and ChIP assays were conducted in human cervical tissues and stromal cells in culture to analyze transcription factor levels, localization, and binding to specific promoters. **Results**: Microarray analysis of cervical stromal tissues from pregnant women before and during labor revealed that a number of genes known to stabilize the transcription factor HIF-1α were upregulated during cervical ripening (e.g., thrombopoetin, 6.2-fold and granulocyte colony-stimulating factor, 13.6-fold). HIF-1α mRNA levels were increased significantly after cervical ripening (5.3±0.6 (n=6) compared with 1.2±0.02 (n=9) units/18S before ripening, $P < 0.01$), and HIF-1α was stabilized and re-localized to the nucleus. Hypoxia- or $CoCl_2$-induced upregulation of HIF-1α resulted in repression of MITF (mRNA and protein) and induction of IL-8 gene expression in a time- and dose-dependent manner in cervical stromal cells. Adenovirus-mediated ectopic expression of MiTF blocked HIF-1α-induced upregulation of IL-8 and rescued loss of MiTF binding to both IL-8 and MiTF promoters in vivo. Interestingly, in addition to HIF-1α, hypoxia or $CoCl_2$ also induced COX-2 gene expression in these cells (from 0.9 ± 0.05 to 5.5 ± 0.2, 150 μM, 6h, $P<0.01$). Treatment with PGE2 dose-and time- dependently resulted in loss of MiTF, and these effects were inhibited significantly by the EP2 receptor antagonist AH6809 (10 μM). **Conclusions**: Taken together, these data suggest that stabilization of the transcription factor HIF-1α results in upregulation of COX-2, prostaglandin-induced loss of MiTF, absence of MiTF binding to the IL-8 promoter, and thereby upregulation of IL-8 gene expression. The results support a pivotal role for HIF-1α in the initiation of cervical ripening in women.

O-034

The Kir 7.1 Blocker VU590 Causes Profound Contractions in Human Myometrium. Elizabeth Bailey,[1] Siobhan Quenby,[1] Steve Thornton,[2] Andrew M Blanks.[1] *[1]Reproductive Health, Warwick Medical School, United Kingdom; [2]Dean's Office, Penninsula School of Medicine, United Kingdom.*

Background

We have previously demonstrated that the Kir7.1 inhibitor VU590 has profound effects on uterine contractility in mice. This effect is mediated by maintaining the myometrial action potential in a sustained depolarised state. The effect of VU590 in mice is gestation dependent, commensurate with a gestation dependent decrease in Kir7.1. In this study we tested whether the effect of VU590 in mice is replicated in the human to assess it's therapeutic value as a treatment for post-partum haemorrhage.

Aim

To determine the effect of VU590 on human myometrial contractility.

Methods

Biopsies of human myometrium (n=20) were taken at term not in labour (G 38-40wks) caesarean section with informed consent. Strips were mounted in physiological saline with 20mN applied tension and equilibrated for 90mins. VU590 alone (1 μM – 100 μM) or VU590 combined with 1 nM oxytocin(OT) was added to spontaneously active strips. Analysis was split in 3 phases (1) first five contractions following dosing, (2) prolonged contraction phase and (3) remaining contractions. For each phase the activity integral (AI), maximal force (MF) and contraction duration (CD) per contraction was measured and expressed as a percentage of mean baseline activity before dose. Results are expressed as ± SEM. Significance was determined by Wilcoxon signed-rank test.

Results

Phase (1) VU590 alone and with OT resulted in a time-dependent increase in AI in doses up 10 μM with maximal increase of 217±32 % of pre-dose contractions at VU590 1 μM + 1 nM OT at contraction 5 ($P<0.001$ n=9). Phase (2) prolonged contraction consisted of a regular upstroke followed by a gradually diminishing contraction with CD of 6.7±1.9 hrs (n=7) at 10 μM VU590 and 3.3±1.2 hrs (n=5) at 10 μM VU590 + 1 nM OT. Despite reduced CD the AI increased 2256±961 % ($P<0.001$, n=6) at 10 μM + 1 nM OT vs 1139±305% ($P<0.001$, n=7) at 10 μM VU590 alone. In phase (3) remaining contractions in VU590 alone saw maximal increase in AI from pre-dose at 203±27% ($P<0.05$, n=9) at 3 μM VU590 increasing to 281±33% ($P<0.05$, n=10) when combined with 1 nM OT.

Conclusion

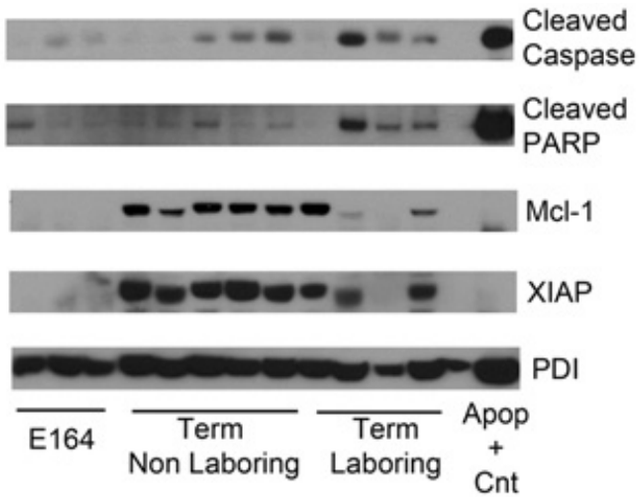
VU590 augments spontaneous contractions profoundly in human myometrium in vitro. VU590-like compounds could have potential therapeutic benefits in the treatment of post-partum haemorrhage. Further investigation is needed into the specific mechanism of action of VU590.

O-035

Apoptotic Caspase-3 Action in Laboring and Post-Partum Baboon and Mouse Uteri. Jason Marks,[1] Peter W Nathanielsz,[2] Jeyasuria Pancharatnam,[1] Jennifer C Condon.[1] *[1]Obstetrics and Gynecology, University of Pittsburgh; [2]Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio.*

Introduction: A tocolytic non-apoptotic role for uterine caspase-3 (CASP3) during pregnancy has been described by our group (1). CASP3 disables the uterine myocyte contractile architecture rendering the pregnant uterus relatively quiescent during late gestation. The uterus retains its non-apoptotic status despite elevated CASP3 levels through increased anti-apoptotic signaling (2).

As term approaches CASP3 levels decline permitting the pregnant uterus to regain its contractile ability. However we have observed a reestablishment of an active CASP3 profile in the laboring (IL) pregnant mouse and baboon uteri and in the post-partum (PPT) mouse uterus.
Hypothesis: Increased CASP3 in IL and PPT uteri is associated with apoptotic events that may modulate PPT uterine involution.
Methods: Pregnant mouse uteri from E18 to Day 2 PPT and fundal myometrium from term pregnant baboons both IL and not in labor (NIL) were examined by immunoblot analysis for active CASP3, apoptotic and non-apoptotic signaling. PARP cleavage was utilized as an indicator of apoptotic CASP3 action.
Results: Distinct re-activation of CASP3 was observed in the IL and PPT uteri. In contrast to term NIL and earlier gestational time points the IL and PPT CASP3 activation was associated with increased PARP cleavage. These events were related to a dramatic decline in the uterine anti-apoptotic factors MCL1 and XIAP in the IL and PPT uteri.



Conclusion: A distinct switch in uterine CASP3 function from non-apoptotic to apoptotic occurred with the onset of labor and delivery. WE speculate that activation of an apoptotic CAP3 profile in the IL and PPT uteri as a result of decreased anti-apoptotic signaling likely signifies the onset of rapid PPT uterine remodeling. Understanding these events may help us identify the potential mechanisms regulating involution abnormalities and postpartum atony which are important obstetrical complications in women.
References: (1) Jeyasuria P. Biol Reprod. 2009 May; 80(5):928-34. (2) Jeyasuria P. Biol Reprod. 2011 Aug;85(2):417-24.

O-036

Prostaglandin (PG) F2α Regulates the Expression of Uterine Activation Proteins (UAPs) in Upper and Lower Segment Primary Human Myometrial Smooth Muscle Cells (HMSMC). Chen Xu,[1,2] Fang Xin,[2] Andrea Mosher,[3,4] Stephen Wood,[3] Donna M Slater,[3,4] Xin Ni,[1] David M Olson.[2] *[1]Physiology, Second Military Medical Uni., Shanghai, China; [2]OB/GYN, Pediatrics & Physiology, Uni. of Alberta, Edmonton, AB, Canada; [3]OB/GYN, Uni. of Calgary, Calgary, AB, Canada; [4]Physiology & Pharmacology, Uni. of Calgary, Calgary, AB, Canada.*
Background: The myometrial switch from pregnancy to delivery involves changes in expression of numerous proteins. It is unlikely that these changes are uniform throughout the myometrium as the upper segment (US) is histologically and functionally different than the lower segment (LS). While steroid hormones can regulate the switch, the involvement of other regulators of UAPs is also likely. One of these is PGF2α, a contractile agonist.
Hypothesis: PGF2α regulates UAPs expression similarly in US and LS HMSMC. **Methods:** Term-non-labor, HMSMC, cultured from LS and US biopsies, were challenged with PGF2α (0.01-10mM) for 6 & 24h (n=4-7). Real time PCR or Western Blot assessed abundance of four proxy UAPs.
Results: PGF2α signficantly up-regulated connexin-43 (CX43) and PG endoperoxide H synthase (PGHS-2) mRNA and protein expression by 1.5-2 fold ($p<0.05$) similarly in LS and US. The expression of the PGF2α receptor, FP, was greater in US than LS (~2 fold, $p<0.05$), but increasing concentrations of PGF2α suppressed FP abundance ($p<0.01$) in US and LS. PGF2α effects on oxytocin receptor (OTR) demonstrated the only real differences. In LS, PGF2α doubled OTR abundance ($p<0.01$), whereas it decreased OTR in US. To test possible mediator action of other PGs, PGD2, PGE2, PGI2 and TX stimulated CX43; stimulated PGHS-2 only in LS weakly; and had little or no effect on OTR. But none down regulated FP. Indomethacin (10^{-6}M) co-incubation did not alter the quality of PGF2α actions, only the quantity suggesting exogenous PGF2α stimulated endogenous PGs synthesis. Specific antagonists of FP attenuated PGF2α actions. PGF2α effects on UAPs are not unique in that interleukin (IL)-1β (1ng/mL) mimicked PGF2α and was additive when co-incubated with PGF2α (1mM) for UAPs except FP where IL-1β effect was opposite and blocked PGF2α-induced attenuation.
Conclusion and Significance: These novel data demonstrate that PGF2α regulates UAPs similarly in LS and US (save OTR in US), preparing the uterus for delivery. Its actions are mimicked by and additive with IL-1β, which rescues FP expression, thereby preserving the PGF2α effect. NSFC, CIHR, and AIHS PreHot.

O-037

Human Placental Multipotent Mesenchymal Stromal Cells Involve in Placental Angiogenesis Via PDGF-BB and STAT3 Pathway. Chie-Pein Chen,[1,2] Shu-Hsiang Liu,[2] Jian-Pei Huang,[1] Chia-Yu Chen.[2] *[1]Division of High Risk Pregnancy, Mackay Memorial Hospital, Taipei, Taiwan; [2]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan.*
Objective: Interactions between endothelial cells and human placental multipotent mesenchymal stromal cells (hPMSCs) in placental angiogenesis are not known. We aim to assess the smooth muscle cell differentiation capability of hPMSCs and determine how endothelial cells recruit hPMSCs to participate in vessel formation.
Methods: hPMSCs and endothelial cells were isolated from term placentas and umbilical veins. hPMSCs were assessed for phenotype and mutilineage capacity by flow cytometry and immunostaining. hPMSCs were induced to differentiate into smooth muscle cells in induction culture condition and matrix substrates. Endothelial cells were co-cultured with hPMSCs in growth factor-reducing Matrigel for angiogenesis assay by cumulative capillary-like tube length measurement. Endothelial cell conditioned medium was assayed for PDGF-BB and used for hPMSC migration assay. Western blot, STAT3 DNA-binding activity assay, and STAT3 siRNA were used for PDGF-BB signaling study in hPMSCs.
Results: hPMSCs were positive for the multipotent mesenchymal stromal cell markers including CD13, CD29, CD44, CD54, CD73, CD90, CD105, CD166, SSEA-4 and Oct-4. hPMSCs were capable of differentiation into osteocytes, adipocytes, and smooth muscle cells. hPMSCs cultured on collagen I or IV enhanced smooth muscle differentiation than that on fibronectin or laminin. The hPMSCs can incorporate into endothelial cells with tube formation and promote endothelial cells forming capillary-like network than that of endothelial cells alone (tube length: 12024.1±960.1 vs. 9404.2±584.7 pixels, $p<0.001$). The capillary-like tube formation was significantly reduced by hPMSCs pretreated with PDGFR-β blocking antibody, but not PDGFR-α blocking antibody or isotype IgG ($p<0.001$). PDGF-BB activated the transcriptional activity of STAT3 in hPMSCs. Endothelial cells expressed PDGF-BB. Endothelial cell conditioned medium induced hPMSC migration, which was inhibited by hPMSCs transfected by STAT3 siRNA or pretreated by PDGFR-β blocking antibody, but not PDGFR-α blocking antibody or isotype IgG ($p<0.01$).
Conclusions: These observations reveal the endothelial cell-hPMSC interactions that occur during vessel development of placenta. Endothelial cells-derived PDGF-BB may recruit hPMSCs involved in vascular development via STAT3 activation and hPMSC migartion.

O-038

EDVs Are Nanoparticles Promoting Targeted Delivery of Doxorubicin to Human Placental Cells Xenografted In Vivo, Causing Regression: A Potential Therapeutic for Ectopic Pregnancy. TJ Kaitu'u-Lino,[1] S Pattison,[2] L Ye,[1] JA MacDiarmid,[2] H Brahmbhatt,[2] U Nilsson,[1] T Johns,[1] S Tong.[1] *[1]Mercy Hospital for Women, Dept of O&G, University of Melbourne, Heidelberg, Victoria, Australia; [2]EnGeneIC, Pty Ltd, Sydney, NSW, Australia.*
Background:
1-2% of pregnancies are ectopic. Methotrexate resolves small ectopics, but most are too large and require surgery.
EnGeneIC Delivery Vehicles (EDVs) are nanoparticles that deliver drugs to target tissues. They are sterile spheres of 400nm diameter, composed of the lipid bilayer of cell walls. Drugs (1,2) can be loaded into EDVs and antibodies attached to the surface recognising epitopes expressed on target tissues. Upon intravenous administration, EDVs stably hold drugs until they reach the tissue of interest where they are endocytosed, and the drug released.

Objective:
To examine whether EGFR targeted (high placental expression) EDVs loaded with doxorubicin regress placental tissue xenografted in mice.
Methods:
JEG3 cells were xenografted into SCID mice. EDVs (3 concentrations, figure 1), naked doxorubicin (equivalent to the highest EDV concentration), or saline alone, were administered intravenously every 2 days from day 4 post-xenograft.
Results:
JEG3 cells treated with doxorubicin were killed in a dose dependent manner in vitro confirming doxorubicin as an appropriate drug to load EDVs. Immunofluoresence showed JEG3 cells highly express EGFR, suggesting this an appropriate EDV target. JEG3 xenografts were vascular, proliferative (ki67 staining) and stained strongly for syncytin.
Administering EDVs to mice with JEG3 xenografts significantly decreased tumour volumes by d11 ($p \leq 0.05$) at three concentrations tested compared to naked doxorubicin or saline alone (Figure 1). Further functional studies are in progress.

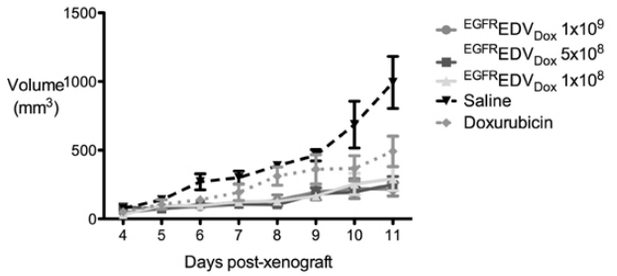


Figure 1: Placental xenograft volumes in SCID mice treated with EGFR targeted doxorubicin containing EDVs, saline alone or naked doxorubicin. n=3-4 animals/group, data expressed as meanSEM.

Conclusion:
EDVs loaded with doxorubicin targeting EGFR significantly inhibited growth of placental cells in vivo. This may be a novel approach to treat ectopic pregnancy and other disorders of trophoblast overgrowth (molar pregnancy, choriocarcinoma and placenta accreta).
References
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O-039

Effects of Placental VEGFA Deficiency on Pregnancy in Mice. Han Li,[1,2] Dawei Qu,[1] Hoon-Ki Sung,[1] Andras Nagy,[1,4] S Lee Adamson.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital; [2]Physiology, University of Toronto; [3]Obstetrics and Gynecology, University of Toronto; [4]Molecular Genetics, University of Toronto, Toronto, ON, Canada.*
Introduction: VEGFA is expressed in the placenta and increases in the maternal circulation during pregnancy, but its role is uncertain. In the mouse placenta, *Vegfa* mRNA is most abundant in the spongiotrophoblast layer (SP). Like the cell columns of the human placenta, this layer is devoid of endothelial cells. Given that SP is perfused by blood returning to the maternal circulation, we hypothesized that disrupting spongiotrophoblast VEGFA (SP-VEGFA) primarily affects maternal functions without directly affecting the conceptuses.
Methods: To disrupt SP-VEGFA, we bred homozygous (HM) *Tpbp-Cre* males that express Cre specifically in SP with HM *Vegfa-loxP* females to obtain dams (n=10) carrying 100% conceptuses with single deletion of SP-*Vegfa* (SD_{100}). We also bred males that were both heterozygous for *Tpbp-Cre* and HM for *Vegf-loxP* with HM *Vegf-loxP* females to obtain dams (n=8) carrying 50% conceptuses with double deletion of SP-*Vegfa* (DD_{50}). HM *Vegfa-loxP* females bred with wild type males were used as controls (n=7). At E17.5 (near term), we measured maternal plasma and placental VEGFA by ELISA, maternal arterial blood pressure by catheter, and recorded embryo viability and body weight. All changes reported here were significant ($P<0.05$ by ANOVA).
Results: As anticipated, SP-VEGFA protein was decreased in SD_{100} and DD_{50} pregnancies, by 27% and 50% respectively. Surprisingly, in SD_{100} pregnancies, maternal circulating VEGFA increased by 20% accompanied by a 15% decrease in blood pressure with no impairment in embryo growth. In DD_{50} pregnancies, 17% of conceptuses had been reabsorbed (18 of 108 implantation sites) and body weight of surviving fetuses was decreased by 7%. Wild type and SP-VEGFA-altered embryos from DD_{50} pregnancies were similarly affected.
Conclusion: Results show that maternal function was indirectly affected by spongiotrophoblast VEGFA deficiency as shown by the paradoxical increase of maternal plasma VEGFA, and the decrease in maternal blood pressure with single deletion of spongiotrophoblast *Vegfa* in all conceptuses. An indirect effect on maternal function is also suggested by impaired conceptus viability and growth that is independent of genotype as a consequence of double deletion of spongiotrophoblast *Vegfa* from half the litter.
Supported by CIHR operating grant MOP-93618.

O-040

Fetal Endothelial Progenitor Cells Transmigrate to the Maternal Circulation and Contribute to the Vasculature of the Pregnant Uterus. P Sipos,[1] W Rens,[2] H Schlecht,[1] F Fen,[3] M Wareing,[1] P Baker,[3] S Davidge,[3] C Sibley,[1] I Crocker.[1] *[1]MFHRC, UManches; [2]DVM, UCambr; [3]O&G, UAlberta.*
Introduction: Uterine blood flow increases 15 fold in pregnancy, requiring robust expansion of its microvasculature. Angiogenesis, driven by mature endothelial cells, may be insufficient to meet this expansion and Endothelial Progenitor Cells (EPC) could play a role. The most potent EPC subtype, Endothelial Colony Forming Cells (ECFC), is more proficient in the fetus than adult. We previously demonstrated placental sequestration of fetal ECFCs. Here we hypothesize that fetal ECFCs migrate across the placenta, circulate in maternal blood and home to sites of vasculogenesis, primarily the pregnant uterus.
Methods: (1) Transgenic male mice, ubiquitously expressing enhanced Green Fluorescent Protein (eGFP), were mated with wild type females. On D18.5, uteri were examined with optical imager/fluorescence microscopy for presence of eGFP-positive fetal endothelial cells. (2) Transduced human fetal ECFCs and HUVEC controls expressing eGFP or LacZ, were in vivo transplanted into the circulation of murine (NOD/SCID) fetuses at D15 of pregnancy by ultrasound guided intra-cardiac injection. On D18.5, uteri were examined for integrated ECFCs expressing tight/gap junctions. (3) The male specific SRY gene was quantified by RT-QPCR in microvessels from human lower segment uterine biopsies, obtained at Caesarean section of women with male babies. Cross sections of similar human vessels were hybridized in-situ for the Y-chromosome, to specify fetal cell locations.
Results: (1) eGFP-expressing fetal endothelial-like cells were located in the endothelium of murine uterine vasculature (n=7 mice). (2) Human ECFCs transplanted into the murine fetal circulation integrated into the vasculature of the maternal uterus (n=8), while control HUVECs were absent in all cases (n=4). (3) RT-QPCR detected SRY gene in 50% of human uterine vessels (n=12 pregnancies). The number of copies was used to calculate the presence of 246±45 (mean±SEM) fetal cells/mm2 of endothelium, covering around 10% of vessel walls. FISH detected fetal cells in the lining of these vessels (n=4 pregnancies).
Conclusion: Our data support the hypothesis that fetal ECFCs contribute to vasculogenesis in the pregnant uterus. We speculate that these cells may provide long-term assistance with maternal vascular repair, but equally their failure could underscore pregnancy complications of utero-placental dysfunction.

O-041

Placental Mammalian Target of Rapamycin Signaling Is Influenced by Maternal High BMI in a Sex Dependent Manner. Francesca Gaccioli, Thomas Jansson, Theresa L Powell. *Dept of Ob/Gyn, Center for Pregnancy and Newborn Research, University of Texas Health Science Center, San Antonio, TX, USA.*
OBJECTIVES: Fetal growth is different in female and male fetuses with girls growing slower and having lower birth weights than boys. The molecular mechanism behind these findings is not known, however sex specific differences in placental function have been implicated. The mammalian target of rapamycin (mTOR) signaling pathway stimulates placental nutrient transport and is involved in the regulation of fetal growth. We hypothesized that: i) placental mTOR activity is lower in female than in male placentas; ii) maternal overweight/obesity differentially modulates mTOR activity depending on fetal sex.
METHODS: Placentas were obtained from 46 normal pregnancies at term, 19 women with normal BMI (21.9 ± 0.51; birth weight: 3345 ± 59 g) and 27 women with high BMI (33.1 ± 0.99; birth weight: 3635 ± 80 g). Phosphorylation of ribosomal protein S6 (rpS6) at Ser235/236 and 4E-binding protein 1 (4E-BP1) at Thr36/47 and Thr70, readouts of mTOR Complex 1 (mTORC1) activity, was determined by Western blot analysis. Akt phosphorylation (Thr308) was used as an indicator for insulin/IGF-I signaling. Group differences were analyzed by one-way ANOVA (Tukey post hoc test).
RESULTS: In lean women phosphorylation of rpS6 was not significantly different between males and females. In response to overweight/obesity the phosphorylation of rpS6 increased 2.2-fold ($P<0.05$) in female placentas but not in male placentas. This leads to a significantly higher rpS6 phosphorylation

in female compared to male placentas in high BMI pregnancies. The phosphorylation of placental rpS6 did not differ between lean and high BMI women when males and females were analyzed together. As we previously reported, phosphorylation of placental 4E-BP1 was significantly increased in high BMI compared to normal BMI women both at Thr36/47 and at Thr70. However, fetal sex did not influence 4E-BP1 phosphorylation. Similarly, we found no significant effect of fetal sex on Akt phosphorylation in the same placentas.
CONCLUSION: Fetal sex has a marked influence on the regulation of placental rpS6 activity in response to maternal overweight/obesity. This effect is specific to rpS6 and is not caused by differences in insulin/IGF-1 signaling in male and female placentas. We speculate that fetal sex influences mTORC1 activity contributing to sexual dimorphism in placental function, such as nutrient transport capacity.

O-042

Protection of Mitochondrial Function in the Placenta of the Female Fetus Exposed to the Obese Maternal Environment. James Mele, Alina Maloyan, Leslie Myatt. *CPNR, OB/GYN, UTHSCSA, San Antonio, TX, USA.*
Obesity in pregnancy has immediate complications for mother and fetus during gestation but also long-term health consequences for the offspring, including cardiovascular disease and metabolic syndrome. The pathologic consequences of maternal obesity differ between male and female offspring. We have recently shown reduced mitochondrial respiration in the placentas from obese (OB) and overweight (OW) vs. lean (LN) women. However, there is a considerable compensation in mitochondria from placentas of a female fetus including sustained energy metabolism and preservation of ATP. Mitochondria undergo frequent division, fusion, and trafficking, which are critical events for proper function. Therefore, we hypothesized that mitochondrial protection in female placentas is achieved via activation of these dynamic processes. We collected placentas by c-section (no labor) at term from LN (BMI< 25), OW (BMI=25-29) and OB (BMI=30-45) women with either a male or female fetus (n=4/group). Using focused Human Mitochondria PCR arrays (SA Bioscience) we examined expression of 84 genes involved in trafficking, mitodynamics and cell death. Nine genes were significantly increased in female LN group vs. male LN suggesting an intrinsic difference between male and female placentas. These included mitofusin2, outer and inner mitochondrial membrane transport proteins TIMM17A and IMMP2L and 5 members of mitochondrial carrier family SLC25A. The increase in BMI had much more pronounced effect on female placentas compared to male: 34 genes were increased in female OW and OB groups versus only 5 in males ($p<0.005$). Among the 34 genes are mitochondrial fusion proteins (MFN2 and OPA1), which were found to be critical in protection of mitochondrial function, and uncoupling protein 3 (UCP3), a mitochondrial carrier, which protects mitochondria against oxidative damage. The mitochondrial trafficking protein Rho GTPase 2 (RHOT2) was 2-fold higher and Carnitine palmitoyltransferase IB (CPT1B), a shuttle protein in beta-oxidation, was 3-fold higher in female OW and OB placentas compared to male. Noticeably, expression of HSP90AA1 was decreased in OW and OB groups independent of sex of the fetus. Thus, activation of mitochondrial fusion, increase in the expression of mitochondrial transporters and uncoupling proteins in female placentas may protect against the adverse effects of obese maternal environment.

O-043

Dynamic Role of Noxa in Regulating Mitochondrial Cell Death and Autophagy in the Human Placenta. A Rolfo,[1,2] L Llano Pedra,[1] T Todros,[2] I Caniggia.[1,3] *[1]Obstet & Gynecol, SLRI, Mount Sinai Hospital, Toronto, Canada; [2]Obstet & Gynecol, University of Turin, Turin, Italy; [3]Physiol, University of Toronto, Toronto, Canada.*
Objective: Autophagy is a cytoprotective mechanism that exerts degradation of damaged cytoplasmic constituents in response to stressors; however when excessive, it can lead to type II cell death. Emerging evidence highlighted a role for BH3-only Noxa in the regulation of autophagy. Noxa also promotes mitochondrial cell death by binding to pro-survival Myeloid Cell Leukaemia 1 (Mcl-1) thus releasing apoptotic Bax. We previously reported Noxa O2-dependent expression during human placenta development, where it regulates trophoblast cell fate by inducing Mcl-1 degradation. As little is known about Noxa and autophagy, herein we investigated Noxa contribution to autophagy in the human placenta.
Methods: First trimester (5-15 weeks, n=27) placentae were used. Noxa mRNA levels were assessed by Real Time PCR. Noxa and microtubule-associated protein 1A/1B-light chain 3 II (LC3II, a marker of autophagy) protein levels were assessed by Western Blot. Noxa vector was generated and overexpressed (OE) in JEG-3 cells under 3% and 20% pO2. Noxa and p62 localization in Noxa OE cells were assessed by immunofluorescence (IF). Since we previously demonstrated that in the human placenta Mcl-1 binds Matador (Mtd) thus regulating trophoblast cell fate, we examined Mtd and Bax localization in Noxa OE cells by IF. Mitochondria and lysosomes were visualized by Mitotracker and Lysotracker probes.
Results: Noxa expression peaked at 5-9 weeks and this correlated with increased autophagy as shown by increased LC3II levels. Noxa OE in JEG-3 cells was accompanied by a significant increase in LC3II ($p<0.05$) levels at both 20% and 3% pO2. IF showed Noxa/lysosome co-localization and increased lysosome activity in Noxa OE cells. Moreover, Noxa OE promoted p62/lysosome co-localization and increased Mtd shuttling to both lysosomes and mitochondria vs controls. No differences were found for Bax localization in Noxa OE cells vs controls.
Conclusions: Our results highlight a novel role for Noxa as a dynamic regulator of both autophagy and mitochondrial cell death in the developing placenta. Increased LC3 II and Noxa/p62/Mtd lysosomal associations are suggestive of Noxa-induced autophagosome formation. Similarly, Noxa-induced Mtd mitochondrial localization indicates a function for this molecule in mitochondrial cell death activation (Supported by CIHR).

O-044

Suppression of Trophoblast Uterine Spiral Artery Remodeling by Estrogen during Baboon Pregnancy: Impact on Uterine and Fetal Blood Flow Dynamics. Graham W Aberdeen,[1] Thomas W Bonagura,[1] Chris R Harman,[1] Gerald J Pepe,[2] Eugene D Albrecht.[1] *[1]Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD, USA; [2]Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA, USA.*
We have previously shown (Bonagura et al., Endocrinology 149:5078, 2008) that extravillous trophoblast invasion and remodeling of the uterine spiral arteries were suppressed by prematurely elevating estrogen levels in the first trimester of baboon pregnancy. In the present study, we determined the impact of this on uterine and umbilical blood flow dynamics. Uterine and umbilical artery blood flow dynamics were determined by Doppler ultrasonography on day 160 of gestation (term is 184 days) before (i.e. basal) and after a 20 min maternal iv infusion of the vasoconstrictor serotonin (8 µg/min/kg BW) to baboons untreated (n=5) or treated with estradiol benzoate (0.35 mg/day, sc, n=6) daily on days 25-59 of gestation which elevated ($P<0.01$) maternal serum estradiol levels from 0.14 ± 0.01 to 0.75 ± 0.03 ng/ml. Maternal mean arterial blood pressure was elevated by 15% ($P<0.01$) while uterine and umbilical artery blood flow dynamics were not altered in the basal state in baboons treated with estradiol. However, uterine artery diastolic notching appeared, umbilical artery pulsatility index reflecting downstream flow impedance was increased ($P<0.01$, repeated measures regression analysis), uterine and umbilical artery volume flow was decreased over 50% ($P<0.05$) and fetal bradycardia elicited (141 ± 2 to 70 ± 9 beats/min, $P<0.001$) after acute administration of serotonin to baboons in which uterine spiral artery remodeling had been suppressed by prematurely elevating estrogen. These results indicate that suppressing uterine artery remodeling by advancing the rise in estrogen from the second to the first trimester disrupted uteroplacental blood flow dynamics and fetal homeostasis after vasochallenge late in gestation. We propose that the low level of estrogen in the first trimester permits aggressive uterine artery remodeling and the rise in estrogen thereafter during normal pregnancy suppresses and thus controls the extent of uterine artery remodeling and consequently uteroplacental blood flow. Supported by NIH R01 HD13294.

O-045

The PPAR-γ Agonist Rosiglitazone Reverses sFLT1 Hyper-Secretion from First Trimester Placental Villi in a GCM1-Dependent Manner. Sascha Drewlo,[1] Fergus McCarthy,[2] Khrystyna Levytska,[1] Louise Kenny,[2] John Kingdom.[1] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada; [2]University College Cork, Anu Research Centre, Ireland.*
Background
Severe preeclampsia [PE] is a hypertensive disorder affecting 2-3% of pregnancies and is associated with reduced utero-placental perfusion and defects in placental development. Ischemic/mal-developed villi increase secretion of the anti-angiogenic protein sFLT1. The molecular mechanisms driving increased production sFLT1 are presently unknown. Differentiation is impaired in severe PE placentas, characterized by reduced expression of the transcription factor GCM1, its downstream fusion partner Syncytin and the systemic vasodilator

Heme Oxygenase 1 [HO1]. We tested the hypothesis that normal syncytial fusion, via the GCM1 axis, represses sFLT1 and is is regulated upstream by PPAR-γ since it regulates labyrinth development upstream of Gcm1 in mice.
Material & Methods
First trimester villous explants [8-12weeks] were cultivated for 48h in hypoxic [3%] or physiologic[8%] pO2 and including exposure to: vehicle [0.5% DMSO,v/v] or 1-100uM Rosiglitazone, a PPAR-γ agonist or 0.05-0.5μM T0070907(T7), a PPAR-γ antagonist. Tissues were fixed and prepared for immuno-histochemistry [FLT1/HO1]. PPAR-γ activation was assessed by CHIP assay. RNA was extracted to monitor GCM1 mRNA using qRTPCR. In parallel, GCM1 was silenced using siRNA. ELISA was used to measure HO1 and sFLT1 expression.
Results
PPAR-γ activation significantly increased both binding to target DNA [+23.5%vs.,n=4] and mRNA expression of GCM1 [5.9±1fold,n=7]. These changes reduced sFLT1 [59.7±9.8%,n=9] and increased HO1 secretion [57.9%±27,n=9]. By contrast T7 reversed all these observations. GCM1 knock-down significantly induced sFLT1 secretion [59.2%±13] and proved GCM1 dependency.
All data $p<0.05$
Conclusions
Physiologic syncytiotrophoblast differentiation via the PPAR-γ GCM1 axis promotes HO-1 expression and represses sFLT1 to mediate cardiovascular adaptation in pregnancy. Repression of the GCM1 axis, as observed in PE placental villi, induces sFLT1 secretion and impairs HO1 expression. Such changes are likely central to the maternal endothelial dysfunction of PE. We present here an oxygen-independent pathway of sFLT1 induction that is reversible via augmentation of the PPAR-γ GCM1 axis. These data offer a powerful pharmacologic insight into the potential for effective drug treatment of severe PE that could prolong gestation and reverse pathogenesis.

O-046

Placental sFlt-1 Production Is Essential for Normal Pregnancy: Relevance to the Mechanisms of Preeclampsia. Nihar R Nayak,[1] Balamurali K Ambati,[2] Sabita Dhal,[1] Maurice L Druzin,[1] Sanjiv S Gambhir,[3] Xiujun Fan.[1] *[1]Ob & Gyn, Stanford University; [2]Ophthalmology, University of Utah; [3]Radiology, Stanford University.*
Preeclampsia (PE) is a major cause of pregnancy-related maternal and fetal morbidity, yet its pathogenesis is still poorly understood. Previous studies have suggested abnormal increase in serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1) contribute to the symptoms of the disease through antagonism of vascular endothelial growth factor (VEGF). However, we present strong evidence that placental sFlt-1 production is essential for normal pregnancy and that overexpression of VEGF in the endometrium stimulates placental sFlt-1 production and induces clinical signs of PE. First, we knocked down sFlt-1 using lentivirus-mediated, placenta-specific expression of a short hairpin RNA targeted specifically against sFlt-1 following our published methods (*Nature* 443:993; *PLoS ONE* 2011:e16348). Complete knockdown of sFlt-1 significantly increased the number of resorption sites and, remarkably, caused numerous focal hemorrhages in placentae, fetuses, and deciduas at the junctional zone. Second, we developed a greatly enhanced method for endometrial gene delivery in the mouse that produced decidua-specific expression of transgenes throughout successive pregnancies. Overexpression of VEGF in the endometrium by this method caused vaginal bleeding starting on day 10 of pregnancy, hemorrhages in placentae and at maternal-fetal junctions, and increased numbers of resorption sites (25.68%) compared to controls (8.62%). To our surprise, all pregnant animals had symptoms of preeclampsia and intrauterine growth restriction. Blood pressure was elevated from day 9 of gestation, dropping to normal levels only after delivery. Circulating and placental sFlt-1 levels were also increased starting day 12. Samples from day 18 showed signs of focal glomerular endotheliosis in kidneys and proteinuria, and fetal weight was significantly reduced. Histological analysis of placentae and fetuses revealed multiple focal hemorrhages. Placentae also had greatly reduced labyrinthine layers, thicker spongiotrophoblast layers and much greater numbers of giant cells. These results indicate that local placental production of sFlt-1 protects placentae and fetuses against harmful effects of excess VEGF production and suggest that VEGF increase itself may trigger the development of PE. Our findings will have an immediate impact on thinking about therapies for PE.

O-047

Mechanisms of Resistance Artery Hydralazine Relaxation. Nicole Maille,[1] Maurizio Mandala,[1,2] Natalia Gokina,[1] George Osol.[1] *[1]Ob/Gyn, Univ of Vermont, Burlington, VT, USA; [2]Cell Biology, Univ of Calabria, Arcavacata di Rende, CS, Calabria, Italy.*
Background: Although hydralazine is commonly used to treat hypertension during pregnancy, little is known about its effects on the smaller resistance arteries that are important in blood pressure regulation.
Objective: To investigate the vasodilatory mechanisms of hydralazine on mesenteric resistance arteries from late pregnant rats by determining its pharmacologic properties, endothelial dependency, and underlying cellular actions.
Methods: Third order mesenteric arteries were dissected from late pregnant (day 20/22) Sprague Dawley female rats (n = 20). Studies were conducted on isolated, pressurized (50 mmHg) vessels that were either intact or mechanically denuded of the endothelium by air perfusion to determine both vasodilatory efficacy (% maximal dilation) and sensitivity (EC_{50}). Arteries were pre-constricted 40-60% with either phenylephrine (Phe) or potassium depolarizing solution (KCl; 30-35 mM) prior to hydralazine (0.1-100 μM) exposure in order to determine if its effects were dependent on vascular smooth muscle (VSM) hyperpolarization. Changes in endothelial cell Ca^{2+} were evaluated by pre-loading the endothelium with Fura-2AM via intralumenal perfusion. To test for nitric oxide (NO) and prostaglandin involvement, vessels were pretreated with a combination of NO synthase inhibitors (L-NNA+L-NAME, both at 100 μM) or indomethacin (10 μM) prior to hydralazine exposure.
Results: Control vessels dilated to hydralazine with an EC_{50} of 2.41±1.18 μM and an efficacy of 53±8.65%. This response was inhibited >90% by KCl preconstriction. There were no detectable elevations in endothelial cell Ca^{2+} following hydralazine exposure and NOS inhibition did not significantly affect vasodilation, however, pretreatment with indomethacin reduced the efficacy of dilation by >80% (to 10±8.5% @ 100 μM; $p < 0.05$) without changing sensitivity. Endothelial denudation abolished vasodilation to lower concentrations of hydralazine (< 30 μM), but was ineffective at higher doses.
Summary: Hydralazine exerts a potent vasodilatory action on mesenteric resistance arteries during pregnancy that is mediated by a combination of endothelial and VSM mechanisms, although endothelial actions are predominant at lower, clinically-relevant concentrations. This effect is primarily carried out by prostaglandins rather than NO through mechanisms that do not involve elevations in endothelial cell Ca^{2+}.
Support: NIH HL79253 and HL73895

O-048

Role of miR210 in Hypoxia-Induced Mitochondrial Dysfunction in Placenta with Preeclampsia. S Muralimanoharan, A Maloyan, J Mele, Leslie Myatt. *CPNR OB-GYN, UTHSCSA, San Antonio, TX, USA.*
Preeclampsia (PE) affects 5–8% of all pregnancies and is associated with a high maternal and fetal morbidity and mortality. Hypoxia is known to have an important role in various physiological processes and pathological conditions, including preeclampsia. A hypoxic microenvironment causes mitochondrial dysfunction and activates various signaling pathways via hypoxia-inducible factor-1a (HIF-1a), but the exact mechanism is unknown. Placental mitochondrial dysfunction has been reported in PE. MicroRNAs (miRNA) are small non-coding RNAs that regulate gene expression through mRNA degradation and translational repression. miR-210 induction in hypoxia is widely accepted as a hallmark of HIF-mediated response and a number of studies have identified increased placental miR-210 during PE. We therefore **hypothesized that the hypoxia-induced placental mitochondrial dysfunction during preeclampsia can be mediated by miR-210**. Placentae were collected from normotensive pregnancies (CTR) and those complicated by PE (n=6 each) after C-section (no labor) at term. Villous tissue from PE showed significantly elevated HIF-1a protein level ($p<0.05$) compared to CTR but with no change in HIF-1a mRNA expression and reduced DNA-binding activity (EMSA). Electron microscopy revealed swollen mitochondria with broken cristea in PE compared to CTR. The activity of mitochondrial complexes I and III were significantly decreased ($p<0.05$) in PE although there was no change in their protein expression. miR-210 expression was 2-fold higher in PE ($p<0.05$). Accordingly, mRNA expression of the miR-210 targets genes ISCU, NDUFA4, and SDHB was reduced by 4.2, 9 and 2.5 folds respectively in PE compared to CTR ($p<0.05$). To understand the role of miR-210 in PE, loss-of-function studies were performed using isolated cytotrophoblast (CT) from normotensive placentas collected after C-section at term. CT that were treated with the iron chelator Desferoxamine (DFO, 200μM, 24h) to mimic hypoxia showed significant ($p<0.01$) increase in miR-210 expression and

reduction in mitochondrial respiration measured by the Seahorse Extracellular Flux Analyzer. In contrast, transfection of CT with miR-210 antagomir (100nM for 48h) was sufficient to prevent the DFO-mediated respiratory deficiency. These data collectively suggest that hypoxia induced placental mitochondria dysfunction during PE may be mediated by increased expression of miR210. Supported by NIH HD075297

O-049

Blocking Gap Junction Phosphorylation in Uterine Artery Endothelium as a Potential Therapy for Preeclampsia. Derek S Boeldt, Mary A Grummer, Ronald R Magness, Fu-Xian Yi, Ian M Bird. *Ob/Gyn, Perinatal Research Labs, Univ Wisconsin Madison, Madison, WI.*

Introduction: We have previously shown that pregnancy enhancement of endothelial function is denoted by increased Ca2+ burst responses and nitric oxide (NO) output in response to 100 uM ATP. The underlying cause is increased cell-cell communication through Cx43 gap junctions. Clinical conditions associated with endothelial dysfunction, such as preeclampsia (PE), are also associated with elevation of vascular endothelial growth factor (VEGF). Pretreatments of cells with 10 ng/ml VEGF-165 results in an impairment of Cx43 function, inhibiting Ca2+ bursting and associated NO output back down to nonpregnant levels.

Objective: Given that VEGF stimulates Erk and Src kinases, we have investigated the effects of VEGF on inhibitory Cx43 phosphorylation at positions s279/282 (Erk site) and y265 (Src site), in the absence and presence of U0126 (Mek/Erk inhibitor) and PP2 (Src inhibitor). We have also examined if U0126 and PP2 can rescue subsequent ATP stimulated Ca2+ burst function from this VEGF inhibition as a proof of concept that targeting these kinases could rescue normal function.

Methods: Primary uterine artery endothelial cells (UAEC) from nonpregnant (NP) and pregnant (P) ewes were grown to 100% confluence on 35 mm glass bottom dishes. Cells were then loaded with Fura-2 and imaged under stimulation with ATP (100uM) for 30 minutes (control). After washing, the same cells were pretreated as below, followed by a second ATP stimulation. Cx43 phosphorylation was assessed by western blot.

Results: VEGF pretreatment blocked ATP-stimulated Ca2+ burst responses (to 79.6% of control $p<0.001$) in PUAEC to a similar extent as Gap27 (Cx43 inhibitory peptide). Cx43 phosphorylation was observed in response to VEGF at sites s279/282 (1.5 fold of control, $p<0.05$) and y265 (1.33 fold, limited data) and was reversed by U0126 and PP2 respectively. VEGF related inhibition of subsequent ATP-stimulated Ca2+ bursts was also fully rescued by U0126 and PP2.

Conclusion: We have confirmed that both Erk and Src mediate inhibition of Cx43 function following VEGF pretreatment. Further, direct targeting of Erk and/or Src can prevent inhibitory phosphorylations and rescue cells back to normal function. This provides proof of concept that targeting Erk and Src inhibitors in future drug screening may provide novel treatments of conditions of endothelial dysfunction such as PE. *Funded by NIH HL079020, T32HD41921, HD38843.*

O-050

Blockade of CD4+ T Cells Prior to Placental Insult, Attenuates Hypertension and Placental sFlt-1 in Response to Placental Ischemia. Sarah Novotny, Kedra Wallace, Pushpinder Dhillon, Janae Moseley, Judith Heath, James N Martin, Jr, Babbette LaMarca. *Obstetrics and Gynecology, University of Mississippi Medical Center.*

OBJECTIVE: Preeclampsia is associated with hypertension, proteinuria, chronic immune activation involving CD4+ T cells, inflammatory cytokines, anti-angiogenic factor sFlt-1 and agonistic autoantibodies. Our laboratory has shown hypertension in the chronic placental ischemia rat model of preeclampsia (RUPP) is associated with CD4+T cell activation, inflammatory cytokines, sFlt-1, and autoantibody production. Our recent studies indicate adoptive transfer of RUPP CD4+ T cells into normal pregnant (NP) rats causes much of the pathophysiology seen in preeclampsia. Therefore, we hypothesize that blockade of CD4+ T cell activation with abatacept (Orencia) would attenuate hypertension in RUPP pregnant rats.

STUDY DESIGN: Four groups of pregnant rats were examined: NP (NP, n=20), Reduced uterine perfusion pressure (RUPP, n=20), NP+orencia (NP+O; n=12) and RUPP+orencia (RUPP+O; n=19). Orencia (250mg/kg) was infused via jugular catheter on day 13 to NP (n=31) rats, 19 of which underwent the RUPP surgical procedure on day 14, as did RUPP controls. On day 18 indwelling carotid catheters were inserted into all groups. On day 19 MAP was analyzed, plasma collected for FACS analysis of CD4+ T cells, and serum collected for ELISA. Placental explants were isolated, cultured and media analyzed for sFlt-1 secretion via ELISA.

RESULTS: MAP increased from 94+/-2 mmHg in NP rats to 123+/-3 mmHg in RUPP rats. This response was attenuated with CD4+ T cell blockade, MAP was 104+/-2 mmHg in RUPP+O, and had no effect in the NP+O (96+/-2 mmHg). Circulating CD4+ T cells increased in RUPP compared to NP, 66%+/-3% and 55.5%+/-2.7% respectively ($p<0.04$) but was attenuated in RUPP+O (54.8+/-2.5%) and was 59+/-4% in NP+O. The twofold increase in TNF alpha seen in RUPPs (277+/-47 pg/ml) was decreased to 80+/-18 pg/ml in RUPPs+O. Placental sFlt-1 secretion at 2 hrs was 186+/-60 in RUPPs, and was decreased to 53 +/-7 pg/ml in RUPP+O. Placental sFlt-1 secretion at 24 hrs was 488+/-61 pg/ml in RUPP placentas but was significantly decreased to 151+/-28 pg/ml in RUPP+O placentas ($P<0.001$).

CONCLUSION: Blockade of CD4+ T cells prior to the initial placental insult attenuated hypertension, TNF alpha, and placental sFlt-1 secretion in RUPP rats, indicating the importance of immune activation in the pathophysiology of hypertension in response to placental ischemia.

O-051

Maternal Serum PAPP-A2 Levels Are Elevated in Early Onset Preeclampsia at Time of Diagnosis and in Early Gestation. Anita Kramer, Camille Hoffman, Anne Lynch, Virginia D Winn. *Obstetrics and Gynecology, University of Colorado, Aurora, CO, USA.*

Background: Preeclampsia (PE) affects 4% of pregnancies and a need for diagnostic and predictive biomarkers for clinical care exists. Our previous studies demonstrated elevated levels of pappalysin-2 (PAPP-A2) levels in placentas from pregnancies complicated by PE compared to controls (Winn et al., 2009). We sought to determine if PAPP-A2 maternal serum levels would be elevated in pregnancies complicated by PE both at the time of diagnosis as well as early in gestation prior to disease onset.

Methods: Maternal sera were obtained with IRB approval from women diagnosed with PE (n=24) and normotensive controls (n=22). Maternal sera samples were also obtained from a cohort of women between 15-20 weeks gestation who later developed PE (n=18) or remained normotensive (n=18). PAPP-A2 immunoblot and ELISA analyses were performed as previously described (Winn et al., 2009; Nishizawa et al. 2008). Data are presented as mean + SE. Statistical analysis was performed using t-test with Welch correction with a p value of <0.05 considered significant.

Results: PAPP-A2 levels as assessed by ELISA were elevated in the serum of women who delivered preterm (37 weeks) diagnosed with PE compared to controls (148±34 vs. 3±2 pg/mL; $p<0.01$). There was no significant difference in the levels of term PE compared to controls (58±13 vs. 33±16; $p=0.14$). Immunoblot analysis provided similar results. Early gestation PAPP-A2 levels, as determined by immunoblot analysis, were increased in women who later developed PE preterm compared to those that remained normotensive but delivered preterm (1.58±0.12 vs. 1.29±0.06 relative units; $p=0.04$). There was no significant difference in PAPP-A2 for those who developed PE at term compared to term controls (1.28±0.12 vs. 1.11±0.12 relative units; $p=0.32$). PAPP-A2 levels in early gestation samples were below the level of detection by ELISA.

Conclusions: Maternal PAPP-A2 serum levels are elevated in preterm PE both at the time of diagnosis and also early in pregnancy. Distinction in PAPP-A2 levels at term was not appreciated. PAPP-A2 is a potential diagnostic and predictive biomarker for early onset PE. The lack of differences noted at term provides additional evidence that term PE may have a different underlying pathophysiology than preterm PE. Larger prospective studies are warranted to determine the utility of PAPP-A2 as a diagnostic and/or predictive biomarker for early onset PE.

O-052

Endothelial Nitric Oxide Synthase (eNOS) Is Uncoupled When It Binds to Sprouty4. Lin Feng, Hong-hai Zhang, Dong-bao Chen. *Dept of Ob/Gyn, Univ of CA, Irvine, CA, USA.*

Introduction: Protein-protein interaction is a critical mechanism for regulating endothelial nitric oxide (NO) synthase (eNOS) function. We have recently shown that the endogenous receptor tyrosine kinase antagonist sprouty (Spry) co-immunoprecipitates (Co-IP) with eNOS in endothelial cells. However, it is unknown if this association is via direct protein-protein interaction and if yes, whether Spry binding affects eNOS function. **Objectives**: To test if Spry proteins bind to eNOS directly to regulate eNOS function and to identify which domain of the Spry proteins mediates its interaction with eNOS. **Methods**: The

mammalian pCS2 plasmids carrying flag-tagged cDNAs encoding full-length mouse Spry4 (Spry4-F) and its N- and C-terminal fragment peptides (Spry4-N and Spry-C, respectively) were constructed for co-transfection studies with an eNOS expression vector into HEK 293T cells. Total cell lysates were prepared for Co-IP and flag pull-down studies. Cells were fixed for Spry and eNOS double immunofluorescence confocal microscopy. The Spry4-F, Spry4-N, Spry4-C, and eNOS cDNAs were inserted into pGEX-4T-3 for preparing recombinant GST-fusion proteins using BL21 *E. coli*. The GST-fusion proteins were purified and used for in vitro Spry-eNOS binding assays. Real-time living cell fluorescence imaging was used for measuring intracellular NO and superoxide production in cells loaded with DAF-2DA and dihydroethdium (DHE), respectively. **Results**: Co-IP and flag pull-down studies showed that eNOS interacts with the Spry4-F and Spry4-C, but not Spry4-N. Double fluorescence imaging also confirmed perinuclear eNOS co-localization with Spry4-F and Spry4-C; whereas Spry4-N was mainly aggregated in the nuclei. In vitro GST-fusion protein binding assays showed that recombinant Spry4-F and Spry4-C, but not Spry4-N, directly bind to eNOS. In 293T cells transfected with eNOS alone, Ca^{2+} ionophore A23187 rapidly stimulated NO without superoxide production. When eNOS was co-transfected with Spry4-F or Spry4-C into 293T cells, A23187 stimulated superoxide but not NO production. Moreover, in primary endothelial cells Spry4-F overexpression also significantly inhibited NO but enhanced superoxide production by stimulation with angiogenic growth factors. **Conclusion**: Spry4 interacts directly with eNOS via a domain in its C-terminus causing eNOS uncoupling to produce superoxide instead of NO (Supported by HL70562 & HL98746).

O-053

Novel Gene-Gene Interactions and the Risk of Neurodevelopmental Delay Following Early Preterm Birth. Erin AS Clark, Maged M Costantine. *Eunice Kennedy Shriver National Institute of Child Health and Human Development, MFMU Network, Bethesda, MD.*

OBJECTIVE: Genetic polymorphisms in inflammation, coagulation, vasoregulation, excitotoxicity and oxidative stress pathways have been associated with neurodevelopmental delay following early preterm birth. We aimed to determine if interactions between genetic susceptibility loci further contribute to the risk of neurodevelopmental abnormalities.

STUDY DESIGN: Secondary case-control analysis of a randomized, controlled trial of magnesium sulfate (MgSO4) before anticipated early preterm birth for prevention of cerebral palsy. Cases were those with mental or psychomotor delay (Bayley MDI or PDI <70) at age 2 years. Controls had normal neurodevelopment. Neonatal DNA was evaluated for eighty polymorphisms (33 genes) in the above pathways using real-time PCR. Initial analyses performed to identify associations with individual loci were stratified by mental and psychomotor delay. Loci with $p<0.05$ on the initial analysis were further evaluated in co-dominant, two-loci genetic models. MgSO4 treatment, maternal race, gestational age at delivery, infant gender and maternal education level were adjusted for in the logistic regression model. Bonferroni correction was used to adjust for multiple comparisons.

RESULTS: In the initial analyses, 3 polymorphisms were associated with mental delay (111 cases and 94 controls) and 12 polymorphisms were associated with psychomotor delay (93 cases and 84 controls). When these were included in the interaction analysis, a two-SNP model (rs1800871/rs1205) involving interleukin-10 (IL10) and C-reactive protein (CRP) was significantly associated with mental delay (P=6.1x10-5, Bonferroni P=0.014), and a two-SNP model (rs1800779/rs952146) involving nitric oxide synthase 3 (NOS3) and IL6 receptor (IL6R) was significantly associated with psychomotor delay (P=3.6 x10-5, Bonferroni P=0.032). MgSO4 exposure was not associated with the outcome in the regression model.

CONCLUSIONS: Novel gene-gene interactions between IL10/CRP and NOS3/IL6R may contribute to the risk of neurodevelopmental delay following early preterm birth.

O-054

Identification of Informative Biomarkers for Threatened Preterm Labour Using Novel Mass Spectrometry Methodologies. Yujing J Heng,[1] Lorne Taylor,[1] Peter Kupchak,[2] Moyez Dharsee,[2] Stephen A Tate,[3] Tony Pawson,[1] Craig E Pennell,[4] Stephen J Lye.[1] *[1]SLRI, Mt Sinai Hospital, Toronto, Canada; [2]OCBN, Toronto, Canada; [3]AB SCIEX, Concord, Canada; [4]School of Women's and Infants' Health, University of Western Australia, Perth, Australia.*

Threatened preterm labour (TPTL) accounts for about 30% of pregnancy-related hospital admissions. Only 5% of these symptomatic women will deliver a premature baby (<37 weeks gestation) within 2-10 days. Increasing evidence demonstrates that peripheral leukocytes can be used to monitor a variety of biochemical and physiological processes occurring in the body. Novel state of the art proteomic capabilities (SWATH) were employed to quantify leukocyte lysate proteins from peripheral blood in women with TPTL with the aim to develop a unique proteomic signature to predict imminent preterm delivery (PTB). A total of 40 samples were analysed, including 16 from women who gave birth preterm and <48 hours of hospital admission, 5 from women who gave birth preterm but >48 hours after admission, and 19 from women who delivered at term; distributions of maternal age, gestational age at presentation, gravidity and parity were equivalent between women who delivered preterm or at term. Samples were subjected to cation-exchange chromatography and on-bead tryptic digestion, and randomly loaded into a TripleTOF 5600 mass spectrometer for SWATH. Data were processed using PeakView. A total of 531 proteins were identified. Forward stepwise logistic regression analyses were employed on both peptide and protein data. Three proteins were highly predictive of PTB within 48 hours of hospital admission, with an area under the receiver operating characteristic curve (ROC AUC) of 0.96, and a sensitivity (SN) of 81% and specificity (SP) of 92% upon application of 10-fold cross validation using the optimal ROC cutoff. A corresponding model using the top peptide for each of the three proteins achieved similar results, with an AUC of 0.96 and cross-validated SN of 81% and SP of 88% at its optimal ROC cutoff. Three other unique proteins (AUC 0.93, cross-validated SN 86% and SP 74%) and their top peptides (AUC 0.92, cross-validated SN 81% and SP 79%) were predictive of PTB at <37 weeks gestation. These data provide putative proteomic signatures predictive of PTB within 48 hours of hospital admission or prior to 37 weeks gestation. These data also highlight the potential to use unique peptides as future diagnostics coupled with mass spectrometry.

O-055

Systemic Increase in the Proportion of Maternal Circulating MCP-1 Activated CD14+CD16- Monocytes Is a Marker of the True Onset of Labor. M Bardou,[1] T Hadi,[1] M Pesant,[1] I Leray,[1] P Sagot,[2] F Lirussi.[1] *[1]CIC-P803, University Hospital, Dijon, France; [2]Obstetrics & Gynecology, University Hospital, Dijon, France.*

Background: Pregnancy and delivery are both complex immune situations, as immune tolerance toward fetus is necessary for successful pregnancy and delivery is an inflammatory process. Monocytes seem to play a pivotal role since MCP-1 that recruits monocytes/macrophages is increased in utero-placental sphere during labor. Study design: We conducted a prospective observational study between April 2009 and October 2010. Peripheral blood samples were obtained from healthy non-pregnant female volunteers (NP, n = 6); third-trimester healthy pregnant patients (HP, n = 18); pre-eclamptic patients (n = 20) and patients with Preterm premature rupture of membranes (PROM, n=46). Study was approved by ethics committee and written informed consent obtained from all patients. Monocyte subpopulations were characterized by flow cytometry with monoclonal antibodies against CD14, CD16, CCR2 and MCP1 in order to investigate the proportion and the level of activation of each monocyte subpopulations, classical (CD14++ CD16-), intermediate (CD14++ CD16+) and non-classical (CD14+ CD16+). Results: Pregnancy, either healthy or complicated, didn't influence the relative proportion of each monocyte subset. Nevertheless, pregnancy, either complicated or not, was associated with a strong diminution in MCP-1 expressing monocytes (79.5%± 19.75 vs 9.3%± 6.8 and 11.9± 8.3, respectively for NP, HP and PROM, P<0.001). Compared to caesarean delivery prior the onset of labor, labor induced a return of the proportion of MCP-1 expressing monocyte to non-pregnant value, both in normal (74.4%±16.9) and PPROM pregnancy (68.4%± 35.6,), despite a significant difference in terms at delivery (39.3±1.4 vs 31.8±3.5 weeks respectively). Finally, CCR-2 (MCP-1 receptor) expression was not modified in monocytes, during labor, but was significantly increased in granulocytes (3646±1080 vs 7338±2718, respectively for non-laboring PROM and laboring PROM, P<0.05). Conclusion: These results suggest that the down regulation of the level of activation of monocytes, via MCP-1 expression might be pivotal for the fetal tolerance within the maternal environment and that the activation of specific monocytes subtypes might be critical for the onset of labor.

O-056

PPROM in Singletons: What Is the Optimal Gestational Age for Delivery?
Ichchha Madan,[1] Roohi Jeelani,[1] Devika Maulik,[2] Rita Zafra,[1] Lindsay Allen,[1] Mike Kruger,[1] Ray Bahado-Singh.[1] *[1]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [2]Department of Obstetrics and Gynecology, University of Missouri-Kansas City, Kansas City, MO, USA.*
Objective: Timing of elective delivery in preterm prelabor rupture of membranes (PPROM) remains a conundrum. Recent meta-analysis by the Cochrane database (Buchanan SL, et al. 2010) reported insufficient evidence to recommend optimal gestational age for delivery. Our objective was to determine the effect of gestational age on maternal and neonatal outcomes in patients with PPROM.
Methods: US birth data from CDC-National Center for Health Statistics natality database for years 2004-2008 was used to identify singleton pregnancies complicated by PPROM with delivery from 32 0/7 - 36 6/7 weeks. Controls were term singletons, 37-40 weeks without PPROM. Maternal and neonatal complications reported by all states were analyzed along with additional outcomes of interest such chorioamnionitis and hyaline membrane disease in newborns reported by a subgroup of states. OR (95% CI) were calculated after adjusting for confounders: preeclampsia, diabetes, chronic hypertension and maternal race. (SPSS-19.0)
Results: There were 134,502 PPROM cases and a similar number of controls. There was a significant decrease in measures of major neonatal complications such as the need for prolonged ventilation and hyaline membrane disease, (see Table 1) with advancing gestational age. The same applied to other morbidities such as low APGAR scores and other complications. Major maternal complications such as abruption either decreased or were not significantly different e.g. chorioamnionitis and cord prolapse, between 34 and 37 weeks.
Conclusion: Given recent concerns around the significant risk of borderline prematurity, we provide newer population-based evidence suggesting that GA beyond 34 weeks might not significantly increase either maternal or newborn risks in PPROM.

Neonatal and Maternal Outcomes

	Adjusted Odds Ratio (Term non: PPROM births as controls)		
	32 Weeks	34 Weeks	37 Weeks
Neonatal Outcomes			
Prolonged Ventilation	11.00 (10.36, 11.69)	6.53 (6.20, 6.87)	2.60 (2.45, 2.75)
Hyaline Membrane Disease	44.27 (35.47, 55.25)	27.52 (22.30, 33.95)	5.03 (3.89, 6.51)
Maternal Outcomes			
Chorioamnionitis	5.69 (4.93, 6.57)	2.82 (2.47, 3.22)	3.15 (2.83, 3.51)
Cord Prolapse	6.05 (4.00, 9.14)	1.72 (1.06, 2.79)	1.56 (1.02, 2.38)
Abruption	14.39 (11.62, 17.81)	6.96 (5.66, 8.56)	1.98 (1.53, 2.57)

O-057

High and Low Maternal Cortisol Levels Are Associated with Preterm Birth. Sindhu K Srinivas, Jamie A Bastek, Anita Weber, Markley N Foreman, Meghan A McShea, Laura M Anglim, Michal A Elovitz. *Maternal Child Health Research Program, OBGYN, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.*
Background: Cortisol is a steroid hormone often released in response to stress. Stress has been associated with an increased risk for preterm birth (PTB). Our objective was to evaluate the association between maternal serum cortisol levels and preterm birth in women with symptoms of preterm labor.
Methods: We performed a cohort study within a large, prospective cohort of women with singleton pregnancies who presented at 22-33 6/7 weeks with signs/symptoms of preterm labor from April 2009 to June 2011. Maternal medical, obstetric history, and delivery information were obtained through chart abstraction. Blood was drawn at the time of presentation with preterm labor symptoms. Cortisol (ng/ml) was measured using R&D System's Parameter Cortisol Assay kit (cat #KGE008). Cortisol was evaluated as a log linear continuous variable and in rough quartiles. Associations between cortisol levels and PTB less than 37 and 34 weeks were calculated using logistic regression. Multivariable logistic regression was used to control for confounders of age and prior preterm birth.
Results: 386 black women were analyzed, among which 29.8% and 14.6% had a preterm birth less than 37 and 34 weeks respectively. Cortisol did not have a linear association with PTB. However, cortisol was observed to have a significant U shaped association with preterm birth such that high and low levels were both associated with PTB <37 weeks (p=0.003). This same U shaped association was observed with PTB <34 weeks (p=0.001). This association persisted after controlling for maternal age and prior preterm birth for both PTB <37 and <34 weeks (p=0.003 & p=0.002 respectively).
Conclusion: Cortisol levels are an indicator of increased stress that has been associated with PTB. There appears to be an association with both high and low cortisol levels and PTB suggesting either different pathways or aberrant regulation of the same pathway. Further research is needed to evaluate the association between cortisol and self reported measures of stress and their combined relationship with PTB.
MOD#21FY08-539 (Elovitz)

O-058

Is Gestational Age at Preterm Premature Rupture of Membranes a Risk Factor for Cerebral Palsy? Federica Accordino,[1] Sara Consonni,[1] Tiziana Fedeli,[2] Gaia Kullman,[3] Agnese Pizzardi,[1] Anna Locatelli.[1] *[1]Obstetrics and Gynecology, University of Milano-Bicocca, Monza, Italy; [2]Neonatology, University of Milano-Bicocca, Monza, Italy; [3]Neuropsychiatry, S. Gerardo Hospital, Monza, Italy.*
Objective: Gestational age (GA) at delivery and spontaneous prematurity are independent risk factors for Cerebral Palsy (CP).We evaluated perinatal risk factors of CP in spontaneous preterm birth with pPROM and with intact membranes and explored whether latency and GA at PROM are independently related to CP.
Methods: All singleton non-anomalous babies born <34.0 weeks from 01-2006 to 06-2010 were prospectively admitted to neurodevelopmental follow-up. Excluded were pregnancies with other complications (i.e. preeclampsia). We compared obstetric, neonatal and placental histology variables in cases of pPROM with that of spontaneous preterm deliveries with intact membranes (PTDs), in reference to the development of CP. Statistical analysis included Chi-square, ANOVA and logistic regression, with P<0.05 considered significant.
Results: In a cohort of 119 neonates after pPROM (n=52) and PTDs (n=67) delivered at 27.6±3.6 weeks, CP occurred in 21(17%), 7 in pPROM and 14 in PTDs. CP was related to GA at PROM (26.9±3.8 in CP vs 29.4±2.6 in no CP,p=0.03) in pPROM group and to GA at delivery (26.9±3.3 in CP vs 29.3±2.3 in no CP,p=0.004) and birth-weight (968±440 in CP vs 1,350±428 in no CP,p=0.004) in PTDs group. Latency period after pPROM was not related to CP (13.4±17.7 in CP vs 5.9±7.9 in no CP,p=0.061). Antenatal administration of betamethasone was not significantly different in cases with and without CP both in pPROM (100% vs 93.3%, p=1) and in sPTD (78% vs 86%,p=0.425). Histological chorionamnionitis was found in 57% babies with CP vs 47% in no CP in pPROM, p=0.7, and in 64% babies with CP vs 41% in no CP in PTDs group, p=0.14. Severe intraventricular haemorrage and periventricular leukomalacia were significantly related to CP, 57.1% in babies with CP vs 0% in no CP, p< 0.001 in pPROM, and 50% in CP vs 5.7% in no CP, p<0.001 in PTDs group. Among antenatal risk factors at logistic regression only GA at PROM in pPROM (p=0.046, 95% CI 0.535, 0.995) and GA at delivery in PTDs independently predicted PCI (p=0.003, 95% CI 0.476, 0.857).
Discussion: Among infants born at <34 weeks the antenatal trigger involved in the damage of CP seems to act at GA of membranes rupture in the pPROM group and at the onset of labor in PTDs.

O-059

Prenatal Vitamin C and E Supplementation Is Associated with a Reduction in Placental Abruption and Preterm Birth in Smokers. Adi Abramovici, Robin E Gandley. *for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network, Bethesda, MD.*
Objective: We evaluated the relationship between prenatal vitamin C/E supplementation and perinatal outcomes by maternal smoking status.
Methods: Secondary analysis of a multicenter trial of vitamin C/E starting at 9-16 weeks in low-risk nulliparous women with singletons. We examined the effect of vitamin supplementation by reported smoker or non-smoker at time of randomization. The primary outcomes were preeclampsia (new onset hypertension and proteinuria) and a composite outcome of severe pregnancy associated hypertension (severe hypertension OR mild or severe hypertension with elevated liver enzymes, elevated serum creatinine, thrombocytopenia, eclamptic seizure, fetal growth restriction, medically indicated preterm birth or perinatal death). Perinatal outcomes included preterm birth and abruption. The Breslow-Day test was used to ascertain whether there was an interaction between smoking status and vitamin supplementation.
Results: Of 9969 women, 4,993 received vitamins C/E and 4,976 received placebo. The prevalence of smoking (15.6% overall; 788 vitamin, 763 placebo) was similar in treatment groups. The analysis of vitamin C/E by smoking status for perinatal outcomes are given (table 1). The effect of prenatal vitamin C/E on the risk preeclampsia or pregnancy associated hypertension composite outcome did not differ by smoking status. Vitamin C/E was protective for placental abruption and preterm birth among smokers.

Conclusion: The effect of vitamin C/E supplementation on preeclampsia/ pregnancy associated hypertension did not differ by smoking status. However, vitamin C/E supplementation was associated with a >90% reduction in placental abruption and >30% reduction in preterm birth among smokers warranting further study.

Table 1. Analysis of Vitamin C/E by Smoking Status for Perinatal Outcomes

	Vitamins %	Placebo%	RR	P value[a]
Preeclampsia				
Smokers	7.9	6.8	1.15 (0.81-1.65)	
Non-smokers	7.0	6.6	1.06 (0.90-1.24)	0.662
Composite Outcome				
Smokers	7.9	7.6	1.04 (0.73-1.46)	
Non-Smokers	5.8	5.4	1.07 (0.90-1.28)	0.863
Placental Abruption				
Smokers	0.1	1.5	0.09 (0.01-0.67)	
Non-smokers	0.6	0.6	0.92 (0.52-1.62)	**0.010***
Preterm Birth (<37 weeks)				
Smokers	10.5	13.9	0.76 (0.58-0.99)	
Non-smokers	10.2	10.0	1.03 (0.90-1.17)	**0.046***
SGA (<10%)				
Smokers	15.1	12.0	1.26 (0.98-1.63)	
Non-smokers	10.8	10.6	1.02 (0.90-1.16)	0.140

[a]Breslow Day test for interaction between vit c/e supplementation and smoking status

O-060

Novel Protective Effects of Pregnancy of Visceral Obesity and Adipose Tissue Inflammation. Silvia MA Pedroni,[1] Sophie Turban,[2] Donald R Dunbar,[3] Tiina Kipari,[4] Vicky King,[1] Nicholas M Morton,[2] Jane E Norman.[1] *[1]Tommy's Centre for Maternal and Fetal Health, MRC Centre for Reproductive Health, University of Edinburgh; [2]Molecular Metabolism Group, Centre for Cardiovascular Sciences (CVS), University of Edinburgh; [3]Bioinformatics Core, CVS, University of Edinburgh; [4]Endocrinology Unit, CVS, University of Edinburgh.*

Objective: Maternal obesity is linked with increased morbidity and mortality for mother and fetus. We hypothesized that obesity in pregnancy would exacerbate adipose tissue inflammation and insulin resistance thus contributing to pathology. To test this we developed a mouse model of obesity in pregnancy and studied the effects on adipose tissue. Methods: 5 week old C57BL/6 female were divided in 4 groups: Control diet fed (C: 11% kcal as fat), pregnant (CP: control diet fed throughout pregnancy), obese (O: 58% kcal as fat for 12 weeks) and obese pregnant (OP: high fat fed throughout pregnancy). Glucose tolerance (GTT) test was conducted at E14.5 and E18.5. Mesenteric fat was obtained at E18.5 and subjected to Affymetrix Mouse Genome MOE 430 2.0 array (n=5 each group). Genes with >±1.5fold and Rank Product<0.05 difference were considered significant and Metacore pathway analysis was validated with qRT-PCR (n=8). Gonadal adipose CD11b+CD11c+ macrophage number (ATM) was determined by FACS. Rbp4 plasma levels were quantified by western blot. Subcutaneous adipocytes were cultured for 6h in DMEM to assess basal lipolysis. Results: OP mice exhibited reduced (-53%) visceral (mesenteric) fat accumulation compared to O ($P<0.001$), higher (+81%) subcutaneous adipocyte basal lipolysis compared to CP ($P<0.05$) and lower (-53%) plasma levels of the insulin-resistance-inducing Rbp4 protein compared to O ($P<0.01$). Relative hyperglycaemia and hyperinsulinemia of OP compared to CP at E14.5 ($P<0.001$) did not worsen in the OP group by late pregnancy (E18.5), suggesting that pregnancy attenuates the obesity phenotype. Microarray analysis revealed gene expression changes suggestive of reduced lipogenic drive (SCD1, Dgat2, FASN, ME1, Rbp4) and reduced inflammation (TNFa and MCP-1) in the OP versus O group. Attenuation of adipose inflammation was functionally confirmed by 45% lower CD11b+CD11c+ ATMs in OP compared to O ($P<0.001$). Conclusion: Pregnancy affords an unexpected protection from high fat diet-induced visceral obesity, through decreased lipid storage and inflammation, and increased lipolysis.

O-061

Statins in the Newborn Period Mature the Lungs. I Bronckers,[1] DP Murphy,[2] BJ Allison,[2] EJ Camm,[2] CM Cross,[2] AD Kane,[2] Y Niu,[2] EA Herrera,[2] FK Lotgering,[1] DA Giussani.[2] *[1]Obstetrics & Gynaecology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Physiology, Development & Neuroscience, University of Cambridge, United Kingdom.*

In addition to cholesterol-lowering effects, statins convey beneficial effects on cardiovascular function by increasing endothelial nitric oxide (NO) bioavailability (Kaesemeyer et al. *J Am Coll Cardiol*. **33(1)**:234, 1999). Surfacing evidence also suggests that statins may similarly improve NO metabolism in the bronchial epithelium, conveying beneficial effects on impaired lung function, for instance in the treatment of asthma (Ahmad et al. Am J Respir Cell Mol Biol **44**:531,2011). However, whether statins are protective in the developing pulmonary system is completely unknown. Here, we investigated whether postnatal treatment with statins conveys any beneficial effects on the pulmonary system in the newborn rat, an established model of lung immaturity.

Methods: One male Wistar rat pup per litter received daily i.p. either saline (n=10) or pravastatin (10 mg/kg; n=9) during P1-6. At P21, fixed and frozen lung tissue was processed for indices of pulmonary maturation.

Results: Relative to controls, postnatal treatment with statins reduced the lung tissue to airspace ratio (1.13 ± 0.09 *vs.* 0.83 ± 0.09), it increased pulmonary elastin (29.74 ± 0.83 *vs.* 32.94 ± 1.14) and the number of secondary crests (1.38 ± 0.15 *vs.* 2.18 ± 0.21) expressed as a percentage of total lung tissue, it increased the expression of pulmonary surfactant protein C (0.17 ± 0.06 *vs.* 0.27 ± 0.06) and antioxidant glutathione peroxidase (0.24 ± 0.05 vs. 0.29 ± 0.04) and it decreased collagen expressed as a percentage of total lung tissue (11.21 ± 0.50 *vs.* 8.88 ± 0.57; all comparisons $P<0.05$).

Conclusions: Postnatal treatment with statins promotes lung maturation in the newborn rat. Statins may offer therapeutic value in the treatment of diseases associated with lung immaturity.

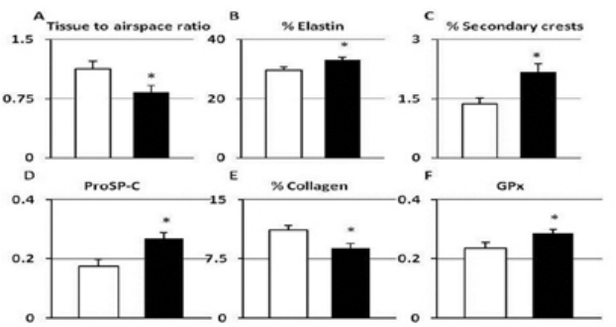


Figure 1. Mean+SEM for the lung tissue to airspace ratio (A), elastin and number of secondary crests, % total lung tissue (B and C), expression of surfactant protein C (D), % collagen total lung tissue (E) and expression of glutathione peroxidase (F) in P21 pups treated daily with i.p saline (white) or with pravastatin (10 mg/kg; black) during P1-P6. *P<0.05, control *vs.* statin, Students *t* test for unpaired data.

O-062

Prediction of Stillbirth and Late-Onset Preeclampsia. Tinnakorn Chaiworapongsa,[1,2] Roberto Romero,[1] Steven J Korzeniewski,[1,2] Juan Pedro Kusanovic,[1] Eleazar Soto,[1,2] Edgar Hernandez-Andrade,[1,2] Zhong Dong,[1] Sonia S Hassan.[1,2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective The prediction of stillbirth and late-onset preeclampsia (PE) is an unmet clinical challenge. Recent evidence suggests that changes in angiogenic and anti-angiogenic factors may assist in the identification of patients at risk for these conditions.

Study design A prospective longitudinal cohort study was conducted; 1269 patients had maternal blood samples obtained between 30-34 weeks and delivered after 34 weeks of gestation. Plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), soluble vascular endothelial growth factor receptor (sVEGFR)-1 and -2 were determined by ELISA. Logistic regression modeling was used to determine the likelihood of PE, stillbirth, and SGA. ROC curves were constructed.

Results The prevalence of PE, stillbirths and SGA was 3.2%(n=40), 0.4%(n=5) and 8.5%(n=108). A low plasma concentration of PlGF/sVEGFR-1 and PlGF/ sEng (< 3rd, 5th and 10th centile of normal) was associated with stillbirth, PE and SGA (see below). Integrating these biomarkers with clinical data (including age, body weight, parity, tobacco, a history of PE and GA at sampling) improved the prediction of late-onset stillbirth and PE from AUC 0.70 and 0.75 to **0.90** and **0.84,** respectively.

Percentile	Stillbirths		PE (n=40)		SGA (n=108)	
	Crude	Adjusted^	Crude	Adjusted*	Crude	Adjusted**
	OR (95% CI)		OR (95% =CI)		OR (95% =CI)	
PlGF/ sVEGFR-1						
<3rd	23.8 (3.2-144)	36 (5.1-256)	6.6 (3.2-13)	6.9 (3.1-15)	1.9 (0.9-3.6)	1.9 (1-3.9)
<5th	40.7 (4.5-367)	59.8 (6-598)	5.5 (2.6-10)	5.4 (2.6-11)	2.5 (1.5-4.2)	2.5 (1.4-4.3)
<10th	21.4 (2.4-192)	30 (3-296)	7.1 (3.7-13)	8.1 (4-16)	2.7 (1.7-4.2)	2.8 (1.8-4.5)
PlGF/sEng						
<3rd	10.7 (1.8-65)	13.7 (2-93)	7.8 (3.8-16)	8 (4-19)	1.9 (1-3.7)	2 (1-4)
<5th	16.2 (2.7-98)	21.6 (3.2-146)	5.6 (2.8-11)	6 (2.8-12)	2.3 (1.3-4)	2.3 (1.3-4.1)
<10th	8.5 (1.4-51)	10.5 (1.6-68)	5.5 (2.9-10)	5.5 (2.9-11)	2.2 (1.4-3.5)	2.3 (1.4-3.6)

^adjusted for GA at sampling; *adjusted for GA at sampling, chronic hypertension, age and pre-pregnancy BMI; **adjusted for GA at sampling, tobacco use and pre-pregnancy BMI.

Conclusions Plasma concentrations of angiogenic/anti-angiogenic factors in the third trimester are strongly associated with stillbirth and PE after 34 weeks. The patients at risk for late-onset stillbirth and PE can be identified with these biomarkers.

O-063

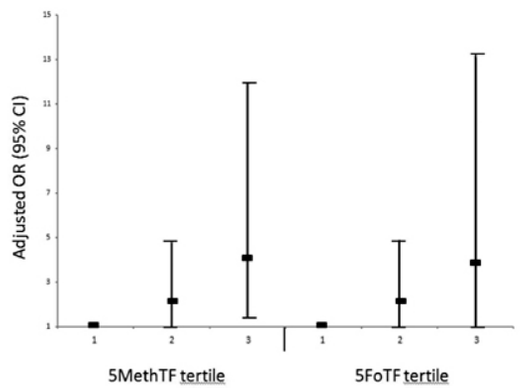
Maternal Folate Species in Early Gestation and Evidence of Histologic Fetal Inflammation. Meredith S Parrott,[1] Lisa M Bodnar,[2] Katherine P Himes,[1] Hyagriv N Simhan.[1] *[1]Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Hospital, Pittsburgh, PA, USA; [2]Epidemiology, University of Pittsburgh School of Public Health, Pittsburgh, PA, USA.*

OBJECTIVE: Placental histopathology can be valuable in linking pregnancy exposures to adverse pregnancy outcomes. Poor maternal folate status is associated with increased risk of preterm birth, a product of many potential etiologies including infection and inflammation. We sought to characterize the association between maternal folate status in early pregnancy and histological evidence of fetal inflammation in the placentas of term deliveries.

STUDY DESIGN: Blood samples drawn from women enrolled in a prospective cohort study at <16wks gestation were assessed for 3 primary folate species: 5-methyltetrahydrofolate (5MeTHF), 5-formyltetrahydrofolate (5FoTHF), and folic acid. Placental histopathologic examination was conducted by a single perinatal pathologist, blinded to clinical circumstances. Fetal inflammation was defined by evidence of a composite of umbilical cord funisitis or arteritis, or chorionic plate chorioamnionitis, vasculitis, or microabscesses. Pathology was correlated with folate species using logistic regression, and limited to only term deliveries.

RESULTS: Evidence of fetal inflammation was present in 24.6% (56/227) of term placentas. Women with the highest tertile of 5MeTHF and 5FoTHF had significantly increased odds of placental fetal inflammation (5MeTHF, OR: 4.1, 95% CI: 1.4- 11.9; 5FoTHF, OR: 3.7, 95% CI: 1.0-13.2), and remained unchanged controlling for confounders.

Figure 1. The odds of evidence of fetal inflammation in the placenta by tertile of folate species



CONCLUSION: The positive relationship observed between high folate status and increased evidence of fetal inflammation in the placenta is interesting. The clinical significance and implication of this association on maternal and fetal wellbeing remains unclear.

O-064

The PAI-1 4G Polymorphism Is Not Associated with an Increased Risk of Adverse Pregnancy Outcome in Asymptomatic Nulliparous Women. Joanne M Said,[1,2] Ruoxin Tsui,[1,2] Anthony J Borg,[1] John R Higgins,[1,2,3] Eric K Moses,[1,2,4] Susan P Walker,[2,5] Paul T Monagle,[6,7,8] Shaun P Brennecke.[1,2] *[1]Perinatal Medicine, The Royal Women's Hospital, Parkville, Australia; [2]Obstetrics & Gynaecology, The University of Melbourne, Parkville, Australia; [3]Anu Research Centre, University College Cork, Ireland; [4]Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia; [5]Obstetrics & Gynaecology, Mercy Hospital for Women, Heidelberg, Australia; [6]Haematology, The Royal Children's Hospital, Parkville, Australia; [7]Paediatrics, The University of Melbourne, Parkville, Australia; [8]Haematology Research, Murdoch Children's Research Institute, Parkville, Australia.*

Plasminogen activator inhibitor type 1 (PAI-1) is an important regulator of fibrinolysis. A common deletion polymorphism which results in a sequence of 4G instead of 5G in the promoter region of the gene is associated with a small increase in the risk of venous thromboembolism. Its potential association with adverse pregnancy events remains controversial. We aimed to assess the impact of the 4G PAI-1 polymorphism on pregnancy outcomes in women who had no prior history of adverse pregnancy outcomes or personal or family history of venous thromboembolism.

Methods: This study represents a secondary investigation of a prior prospective cohort study investigating the association between inherited thrombophilias and adverse pregnancy events in Australian women. Healthy nulliparous women were recruited to this study prior to 22 weeks gestation. Venous blood was obtained and genomic DNA extracted. Genotyping for the 4G/5G PAI-1 gene was performed using Taqman assays in an ABI prism 7700 Sequencer several years after the pregnancy was completed. The primary outcome was a composite comprising severe pre-eclampsia, fetal growth restriction, major placental abruption, stillbirth or neonatal death.

Results: Pregnancy outcome data were available in 1733 women who were genotyped for this polymorphism. The primary composite outcome was experienced by 139 women (8% of the cohort). 459 women (26.5%) were homozygous for the 4G deletion polymorphism, while 890 (51.4%) were heterozygous. Neither homozygosity nor heterozygosity for the PAI-1 4G polymorphism was associated with the primary composite outcome or with the individual pregnancy complications. Conclusion: The PAI-1 4G polymorphism is not associated with an increase in the risk of serious adverse pregnancy events in asymptomatic nulliparous women.

O-065

Maternal Protein Restriction Leads to Elevated Hepatic Endoplasmic Reticulum Stress and Insulin Resistance in Adult Rat Offspring. Gurjeev Sohi, Andrew Revesz, Daniel Hardy. *Children's Health Research Institute, Depts of Ob/Gyn & Physiology/Pharmacology, The University of Western Ontario, London, ON, Canada.*

The World Health Organization has identified insulin resistance and hypercholesterolemia as one of the major symptoms making up the metabolic syndrome. Moreover, epidemiological studies over the last two decades have observed that low birth weight is associated with increased risk of developing the metabolic syndrome, independent of genetics, diet and/or lifestyle. In rodent models, we have previously demonstrated that maternal protein restriction (MPR) results in impaired fetal growth, decreased liver to bodyweight ratio and hypercholesterolemia in adulthood[1], however the underlying mechanisms are not fully elucidated. Given recent studies which demonstrate that endoplasmic reticulum (ER) stress leads to both increased cholesterol and insulin resistance, we ***hypothesized*** that MPR derived offspring are at a higher risk of developing insulin resistance and hypercholesterolemia in adult life due to the presence of elevated ER stress. To address this hypothesis, pregnant Wistar rats were either fed a control 20% (C) or a low 8% protein diet throughout pregnancy and lactation (LP2), or exclusively during pregnancy (LP3). At day 130, glucose tolerance tests indicated impaired insulin resistance in both the LP2 and LP3 offspring. Furthermore, immunoblotting revealed that the LP2 offspring had a significant decrease in the hepatic phosphorylation of AKT1 (Serine 473) and increased levels of p85 protein, both indicative of impaired insulin signaling. This coincided with elevation of established ER stress markers in the liver, including an increase in X box binding protein 1 (XBP-1) mRNA splicing levels and elevated ER chaperones (Glucose regulated protein 94 and 78). This was concomitant with attenuated protein synthesis inhibition, as indicated by increased phosphorylation of elongation initiation factor 2 α (eIF2α) at Serine 51 residue. Interestingly, fetal hepatic GRP94 and 78 protein levels were found elevated in LP offspring at embryonic day 19, suggesting that ER stress may persist from fetal life into adulthood in low birth weight offspring. Future studies will be aimed at uncovering the underlying molecular mechanisms behind the long-term elevation of ER stress in this MPR model of insulin resistance. Supported by CIHR.

1. Sohi *et al. Molecular Endocrinology* 2011, 25(5):785-98

O-066

Maternal-Fetal Genetic Influences of Fetal Growth. Scott W White,[1] Julie A Marsh,[1] Wei Ang,[1] Nicole M Warrington,[1] John P Newnham,[1] Stephen J Lye,[2] Craig E Pennell.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; [2]Samuel Lunenfeld Research Institute, Mt Sinai Hospital, Toronto, Canada.*

Background: Altered fetal growth trajectories are associated with the risk of adult disease: a relationship with both environmental and genetic influences. Fetal genetics (30-40%), maternal genetics and the intrauterine environment all influence fetal growth. The fetus and mother share 50% of their genome and assessing either alone may reveal associations which are surrogate for genetic variants in the other.

Objective: To evaluate associations between known disease-risk genetic variants and altered fetal growth assessing both maternal and fetal genotypes independently and simultaneously.
Methods: DNA was available for 1377 maternal-fetal (MF) pairs in the Western Australian Pregnancy (Raine) Cohort. Fetal growth [abdominal circumference, head circumference and femur length] was assessed on five occasions in half of the cohort. All were assessed for birth weight (BW) and ponderal index (PI). Fifty-eight single nucleotide polymorphisms (SNPs) known to be associated with adult metabolic disease were genotyped in maternal and fetal DNA. Dosage scores were derived describing the number of minor alleles (0-4) of each SNP in MF pairs. Linear mixed effects models assessed longitudinal growth. Multivariate linear regression assessed BW and PI.
Results: Of the 58 SNPs associated with adult metabolic disease, 52 were associated with alterations in fetal growth ($p=1.1x10^{-4}$ to 0.05). More associations were demonstrated when maternal and fetal genotypes were analyzed simultaneously (34 SNPs; $p=2.2x10^{-4}$ to 0.05) or when interactions between MF genotypes (34 SNPs; p=0.002 to 0.05) were considered than when either genotype was considered in isolation (19 SNPs for each). Analyses of dosage scores based on MF pairs identified the greatest number of associations (39 SNPs; $p=1.2x10^{-4}$ to 0.05). Some associations were in concordant directions in mother and fetus whilst several SNPs demonstrated opposing directions of effect.
Conclusion: Knowledge of both maternal and fetal genotypes dramatically increases the ability to uncover genetic associations influencing fetal growth. Using this technique, 60% of genetic variants associated with adult metabolic disease have been shown to influence prenatal growth supporting the theory that there is a significant genetic component in the relationship between antenatal events and adult disease.

O-067

Rescue of Programmed Endothelial Dysfunction in Offspring of Hypoxic Pregnancy: A Comparison between Two Antioxidant Strategies. BJ Allison,[1] JJ Kaandorp,[2] C Lusby,[1] AD Kane,[1] CM Cross,[1] JB Derks,[2] DA Giussani.[1] *[1]Physiology, Development and Neuroscience, University of Cambridge, United Kingdom; [2]Perinatal Center, University Medical Center, Utrecht, Netherlands.*
We and others have shown that prenatal chronic hypoxia programmes endothelial dysfunction in adulthood (Allison et al. *J DOHaD* 2011;**2(1)**: PI-013; Morton et al. *AJP* 2010;**110(4)**:1073). Recent studies have suggested a role for oxidative stress (Allison et al. 2011), triggering an interest in maternal antioxidant therapy. Here, we compared the therapeutic antioxidant effects on programmed endothelial function in adult offspring of hypoxic pregnancy of two interventional strategies: preventing ROS generation with allopurinol versus scavenging ROS once formed with vitamin C.
METHODS: On day 6 of pregnancy, Wistar rats were exposed to either normoxia or hypoxia (13% O2) +/- maternal allopurinol (30mg/kg/day given orally in jelly) or maternal vitamin C (5mg/mL drinking water). Rats were allowed to deliver. At 4 months, second order femoral arteries were isolated from 1 male offspring per litter per group for wire myography. Relaxant responses to methacholine were determined after pre-contraction with phenylephrine. Additional response curves to methacholine were determined following incubation with L-NAME or both L-NAME and indomethacin to assess NO-dependent and NO-independent contributions.
RESULTS: Adult offspring of hypoxic pregnancy showed markedly impaired femoral relaxation to metacholine (Fig. 1 A and B). Maternal vitamin C but not allopurinol rescued the endothelial dysfunction in offspring of hypoxic pregnancy. Hypoxic pregnancy programmed endothelial dysfunction by impairing NO-dependent vasorelaxation (Fig. 1C). Maternal vitamin C rescued programmed endothelial dysfunction by increasing the contribution of NO-independent relaxant pathways.
CONCLUSION: Prenatal chronic hypoxia via oxidative stress programmes endothelial dysfunction in adult offspring. In complicated pregnancy, scavenging ROS once formed rather than preventing their generation via one pathway offers greater therapeutic potential.
BHF, The Royal Society, Ter Meulen and Internationalization Fund

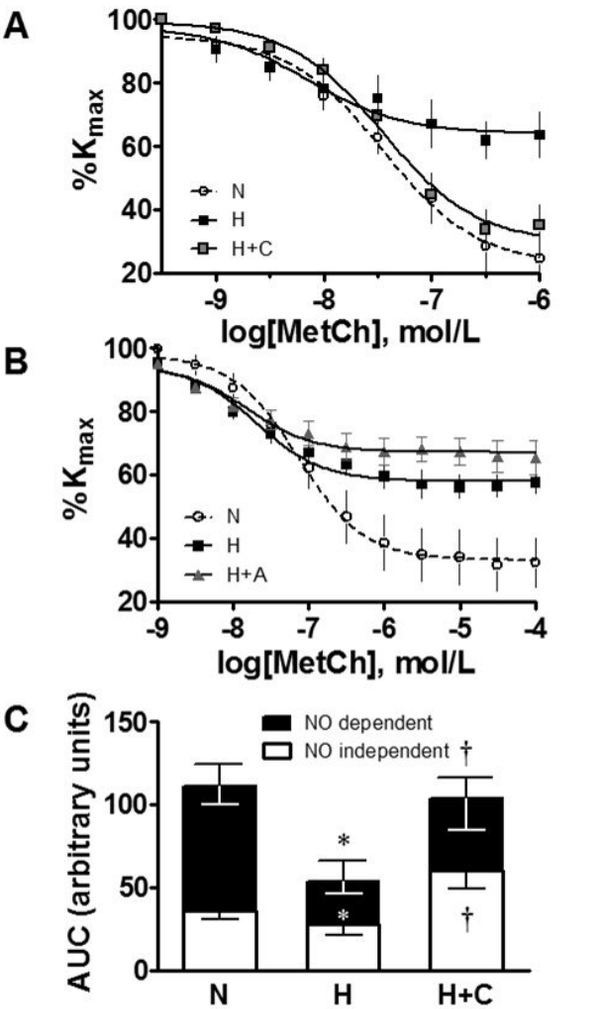


Figure 1. Values are mean+S.E.M. for the concentration-response curve to methacholine (MetCh) in femoral resistance arteries isolated from 4 month adult offspring from the vitamin C (A) or allopurinol (B) experiments. The nitric oxide (NO) dependent and independent components (area under the curve, AUC) of the endothelial- dependent vasorelaxation in the vitamin C experiment is shown in C. Groups are: N= normoxia, n=8; H= Hypoxia, n=8; H+C=Hypoxia+Vitamin C, n=6 and H + A= Hypoxia+Allopurinol, n=7. Significant (P<0.05) differences are: * vs. N, † vs. H, One-Way ANOVA with Tukey Test.

O-068

Maternal Inflammation Programs Newborn TLR4 Pathway through Decrease NFKB Activation and White Blood Indices in Response to Stress. Yuval Ginsberg,[1] Nizar Khatib,[1] Joseph Itskovitz-Eldor,[1] Naomi Lanir,[1] Shimon Pollack,[1] Michael G Ross,[2] Zeev Weiner,[1] Ron Beloosesky.[1] *[1]Ob/Gyn, Rambam Medical Center, Haifa, Israel; [2]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
OBJECTIVE: Maternal gram negative bacterial infections are common during pregnancy. Attachment of lipopolysacchide (LPS) to the monocyte Toll Like Receptor 4 (TLR4) initiates a signal transduction cascade with activation of nuclear factor kappa B (NFKB) and secretion of inflammatory mediators. We have shown previously that acute maternal exposure to LPS at E18 significantly decreases neonatal cytokine responses to LPS. We sought to further determine whether the offspring cascade of immune response is programmed by maternal inflammation.
STUDY DESIGN: Pregnant Sprague Dawley rats (n=4) at 18 days gestation received intraperitoneal injections of saline (Control) or LPS (500 ug/kg). Male and female pups were delivered spontaneously (e21) and allowed to mature.

At postnatal day 24 (p24), the rats were injected with either LPS (100 mg/kg bw) or Granulocyte-macrophage colony-stimulating (GMCSF; 0.2 µg). White blood indices and NFKB activation were determined at base line and three hours following i.p LPS (100 µg/kg) injection. Bone marrow production and secretion of monocytes and granulocytes were determined 3 hours following i.p. Granulocyte-macrophage colony-stimulating (GMCSF) injection.
RESULTS: Newborns of LPS treated dams had significantly higher basal neutrophil and lower lymphocyte percentages than control newborns, though similar monocyte percentage. Following LPS injection, NFKB activation (phosphorylated P65) was significantly lower in the WBCs of newborn of LPS treated dams. In response to GMCSF injection, newborns of LPS treated dams demonstrated higher WBC count (4.6±2.3 vs 3.2±0.6 *1000/ul) and increased monocyte percentage (5.7± 2.2 vs 3.9 ±1.5%) than control newborns.
CONCLUSION: The attenuated serum pro inflammatory cytokine response in the offspring following prenatal maternal exposure to LPS is not due to lower serum percentage of cytokine producing cells (monocytes) or bone marrow reserve monocytes, but rather due to decrease in sensitivity and in the activation of the NFKB following LPS stimulation.

O-069

Effect of Maternal Nutrient Restriction in Developmental Expression of 11β-HSD1in Adipose Tissue: Role of C/EBP. Chunming Guo, Leslie Myatt, Peter W Nathanielsz, Kang Sun. *OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*
Maternal nutrient restriction (MNR) during fetal development may predispose the fetus to chronic diseases in later life. A persistent increase of regeneration of biologically active glucocorticoids (GC) from their inactive counterparts by 11b-hydroxysteroid dehydrogenase type 1 (11b-HSD1) in key metabolic tissues is believed to be fundamental to this developmental programming. However the mechanism underlying this increase of 11b-HSD1 is not resolved. CCAAT enhancer binding proteins (C/EBPs) have been demonstrated to mediate both basal and GC-induced 11b-HSD1 expression in a variety of cell types, and play an important role in adipocyte differentiation.
HYPOTHESIS: MNR in pregnancy increases C/EBP expression thereby leading to a persistent increase of 11b-HSD1 expression in fetal adipose tissue.
METHODS: Pregnant baboons were fed 70% of the control food intake from early gestation (MNR). Fetal peri-renal adipose tissue from control group (n=10, 5 male and 5 female) and MNR group (n=8, 4 male and 4 female) was collected at 165 days of gestation (dG, term: 180dG). Total RNA was extracted and qRT-PCR was performed to measure 11b-HSD1, C/EBPα and β mRNA levels. The role of C/EBPs in 11b-HSD1 expression was further studied in a mouse preadipocyte cell line 3T3-L1.
RESULTS: In the female fetuses, MNR increased 11b-HSD1, C/EBPa and C/EBPb mRNA expression by 3.0, 2.7 and 2.8 fold respectively (P<0.05), but none of these genes changed significantly in the male fetus exposed to MNR. C/EBPa and b mRNA levels positively correlated with 11b-HSD1 mRNA level in both genders (P<0.01). Transfection of 3T3-L1 cells with either C/EBPa or C/EBPb siRNA reduced 11b-HSD1 expression (n=6, P<0.01) compared to transfection with scrambled siRNA. Furthermore, 11b-HSD1, C/EBPa and C/EBPb mRNA and protein levels were increased following differentiation of preadipocyte into adipocyte (n=6; P<0.05).
CONCLUSIONS: Both C/EBPa and C/EBPb are correlated to 11b-HSD1 expression in 3T3-L1cells and fetal adipose tissue of MNR baboon. We also see sexual dimorphism as changes of 11b-HSD1, C/EBPa and C/EBPb expression are only seen in adipose tissue of female fetuses in MNR baboon. The increased 11b-HSD1 expression in female fetal adipose tissue is very likely due to increased C/EBPa and C/EBPb expression, which may regulate the differentiation of preadipocyte.

O-070

Obesity and Hypertension Programmed by Prenatal Glucocorticoid Exposure Is Amplified by a Postnatal High Fat Diet and Attenuated by Omega-3 Supplementation in Male Offspring. Intan S Zulkafli, Peter J Mark, Brendan J Waddell. *School of Anatomy & Human Biology, The University of Western Australia, Perth, Western Australia, Australia.*
Objective: Metabolic syndrome (MS) remains the major cause of heart disease worldwide. A poor fetal environment is known to program symptoms of MS in offspring. In this study, we tested the hypothesis that a prenatal insult of excess glucocorticoid exposure followed by postnatal consumption of a high-fat diet will result in amplification of the programmed metabolic phenotype. We also tested whether high omega-3 fatty acids (Hn-3) supplementation would ameliorate any programmed or amplified effects.
Methods: Wistar rats were either untreated (Con; n=24) or treated with 0.5 µg/ml dexamethasone acetate in drinking water (Dex; n=24) from day 13 of pregnancy until term (day 23). All offspring were cross-fostered to untreated mothers at birth. Male offspring were weaned onto either a standard (Std; n=4-8), high fat, low n-3 (HFLn-3; n=4-8) or high fat, high n-3 (HFHn-3; n=4-8) diet and maintained on these diets until tissues were collected at 6 months of age. Monthly food intake, blood pressure using tail cuff plethysmography and adiposity by DEXA were determined at 6 months of age. Fat accumulation in the liver was assessed by oil-red-O stain.
Results: Dex offspring exhibited a 26% reduction in birth weight (p<0.001). From birth, Con offspring were consistently heavier than Dex offspring (p<0.05; repeated measures ANOVA). Surprisingly, consumption of either high fat diet did not affect growth trajectories compared to those on Std diet. Dex offspring showed delayed puberty onset by 1.2 days (p< 0.05). Caloric intake was increased in animals consuming HFHn-3 diet (p<0.001) with caloric auto-regulation observed in animals consuming HFLn-3 diet. Adiposity was elevated in Dex offspring by 25% (p<0.001; ANOVA) and this effect was corrected by HFHn-3 diet consumption (p<0.001). Programmed hypertension was observed in Dex offspring (p<0.001). This was amplified by HFLn-3 diet (p<0.001, ANOVA) and attenuated by HFHn-3 diet (p<0.001). Animals that consumed the HFLn-3 diet had increased fat accumulation in the liver whilst supplementation with Hn-3 attenuated this outcome.
Conclusions: Prenatal dex treatment resulted in programmed hypertension, increased adiposity and fat accumulation in the liver; effects that were reversed by dietary Hn-3. Postnatal consumption of high fat diet exacerbated programming effects in this cohort.

O-071

Tumorigenic Potential of Oogonial Stem Cells Is Actively Suppressed by the Intraovarian Microenvironment. Dori C Woods, Yvonne AR White, Bo R Rueda, Jonathan L Tilly. *Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.*
Introduction: recent studies indicate that adult mouse ovaries contain mitotically active germline cells (oogonial stem cells or OSCs) that generate oocytes in vitro and in vivo. Purified OSCs undergo self-renewal, express multiple pluripotency markers, exhibit high telomerase activity, and form embryoid bodies in vitro. This apparent multipotency highlights important questions regarding OSC plasticity and pathophysiology.
Objective: to test tumorigenic potential of adult mouse ovary-derived OSCs.
Methods: OSCs were isolated using a VASA antibody-based sorting protocol that employs FACS rather than magnetic beads [Nat Cell Biol 2009 11:631]. The cells were established and expanded in vitro, GFP-transduced and directly injected into ovaries of NOD/SCID mice (1x105 cells, n=10 mice). OSCs were also transplanted subcutaneously (1x105, n=10) or into the ovarian bursal cavity (n=4) of NOD/SCID mice and monitored on a weekly basis. In separate experiments, OSCs were pelleted with phytohemagglutinin without or with dissociated embryonic day 13.5 mouse ovaries (4 ovaries/aggregate, n=3 aggregates per group), and aggregates were grafted under the kidney capsules of NOD/SCID mice. Grafts were retrieved 3 weeks post-transplantation for analysis.
Results: freshly-isolated OSCs did not form tumors after intraovarian or subcutaneous injection. Likewise, intraovarian injection of in-vitro expanded OSCs did not result in tumors; instead, GFP tracing showed the cells formed GFP-positive oocytes enclosed within follicles, consistent with past studies [Nat Cell Biol 2009 11:631]. In contrast, if in-vitro expanded OSCs were injected into the ovarian bursa or under the skin, poorly differentiated tumors formed within 4-6 weeks. Similarly, in-vitro expanded OSCs aggregated without fetal ovaries and placed under the kidney capsules of recipient females rapidly formed tumors; however, this tumorigenic potential was abolished if in-vitro expanded OSCs were aggregated with fetal ovary tissue prior to transplantation.
Conclusion: ovarian microenvironmental signals guide the appropriate differentiation of OSCs into oocytes. However, if OSCs are cut off from these natural cues and misplaced into non-ovarian sites without direct influence of signals emanating from their normal ovarian microenvironment, OSCs gain tumorigenic potential.
Support: NIH R37-AG012279

O-072

MAGE Antigens: Potential Targets for Immunotherapy with Co-Ordinate Expression That Predicts Poor Survival in Epithelial Ovarian Cancer. Sayeema Daudi, Tony Millioto, Amy Beck, Adrienne Groman, Shashikant Lele, Kunle Odunsi. *Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA.*

Objective

The clinical response in epithelial ovarian cancer (EOC) from cytotoxic chemotherapy has reached an apparent plateau, which illustrates a need for alternative therapeutic options. Cancer-testis antigens (CTA) remain a large focus of study, as they appear to be ideal targets for immunotherapy. Melanoma Associated Cancer Testis Antigens (MAGE) are among the best studied cancer testis genes, however, their function remains elusive. To study the potential of the MAGE family CTA for immunotherapy in EOC, we examined the expression, humoral response and the prognostic significance of these antigens.

Methods

207 EOC tissues were examined for MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10 (MAGE-A family) and MAGE-C1 (CT7.1) mRNA expression by reverse transcription-PCR (PCR). MAGE-A4 protein expression was determined by immunohistochemistry (IHC). Sera from a subgroup of the patients were tested for MAGE antibodies by ELISA. The survival outcomes of MAGE expression in EOC were analyzed by a multivariate Cox proportional hazard model, with a 95% confidence limit for the HR.

Results

The overall expression of the MAGE-A family transcripts was demonstrated in 97% of the EOC specimens. The individual frequencies were: 44% A4, 22% A10, 13% CT7.1, 15% A1 and A3. MAGE-A4 expression by IHC was performed due to the high frequency of mRNA expression. Expression of MAGE-A4 by PCR and/or IHC was evident in 37 of the 93 (40%) tissues. The median follow up was 29 months (range: 0-60). MAGE-A3 and CT7.1 expression were associated with a higher risk of death and disease recurrence [HR 1.76, 95% (CI 1.15-2.7), p= 0.01 and HR 1.69, 95% (CI 1.01-2.85), p= 0.047]. Spontaneous humoral immunity to the MAGE-A antigens was present in 16 of 116 (14%) patients whose tumors expressed any of the MAGE-A family antigens. Co-ordinate up-regulation of MAGE-A4 with MAGE-A3 and CT7.1 was apparent in this EOC tumor panel.

Conclusion

Restricted expression of MAGE antigens in normal tissues and expression in a significant proportion of EOC patients, with serological evidence for inherent immunogenicity makes the MAGE antigen family an attractive target for immunotherapy. Moreover, expression of MAGE-A3 and CT7.1 are associated with poor prognosis. Our demonstration of concomitant expression in the MAGE family antigens suggests the induction of a gametogenic program in human ovarian cancer.

O-073

Correlation between Magnetic Resonance Imaging (MRI) and International Federation of Gynecology and Obstetrics (FIGO) Staging in Carcinoma of the Uterine Cervix – A Prospective Pilot Study. Renju S Raj,[1] Cheung Wong,[1] Nitin Shetty,[2] Chandrakanth Shetty,[2] Lavanya Rai.[3] *[1]Ob/Gyn and Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT, USA; [2]Radiodiagnosis, Kasturba Medical College, Manipal, Karnataka, India; [3]Ob/Gyn, Kasturba Medical College, Manipal, Karnataka, India.*

Background

Cervical cancer still remains a leading cause of cancer death in developing world. Thorough pretreatment evaluation and staging of invasive cancer is important as this affects the prognosis and the choice of treatment. FIGO staging for cervical cancer is based mainly on clinical evaluation and has shown not to correlate well with intraoperative findings and surgical pathology. MRI offers a noninvasive means of evaluating the pelvis.

Hypothesis: Staging by MRI does not correlate well with FIGO staging.

Objective: To evaluate the staging by MRI and its correlation with FIGO clinical staging.

Methods: Institutional review board approval was obtained. Newly detected cases of cervix cancer (n=31) underwent clinical (FIGO) staging following which MRI of the pelvis was performed for evaluation of the tumor and its extension. The MRI findings were correlated with clinical findings. There was no surgical pathology correlation as all patients had radiation therapy. Kappa statistics were used to describe the degree of correlation among parameters studied.

Results: Clinical exam and MRI correlated in most of the cases in detecting early parametrial invasion (kappa 0.448±0.07), whereas, clinical exam overdiagnosed parametrial extension to lateral pelvic wall (kappa 0.379±0.107). Early bladder and rectal involvement were picked up only on MRI (Stage IVa). Biopsy proven mucosal involvement is necessary for them to be included in clinical staging. Only MRI picked up silent pelvic bone metastasis (Stage IVb). In addition MRI was able to assess some parameters with prognostic implications which included tumor size/volume and tumor extension to lower uterine segment.

Conclusion

MRI is an excellent imaging modality in cervical cancer for objective demonstration of the tumor extension and the morphologic risk factors. MRI stage showed correlation with FIGO stage in early stages up to IIb. As the stage advanced there were more errors with the FIGO staging. The value of MRI rises in proportion to the volume and stage of the disease. The effects of treatment planning based on MRI findings and its effects on long term patient survival need to be addressed.

O-074

Human Papillomavirus Genotyping in Israeli Jewish Women with Premalignant and Invasive Cervical Cancer. Ido Laskov, Boaz Avidor, Leonor L Trejo, Joseph B Lessing, Dan Grisaru. *Department of Obstetrics and Gynecology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel.*

Background: Israel holds the lowest prevalence of cervical cancer compared to that in other countries of the Western world. However the incidence for premalignant lesions among Israeli Jewish women has become similar to those among women in other Western countries, with a low conversion rate from premalignant cervical lesions to invasive cancer.

Aim: To establish the HPV genotypes distribution among Israeli Jewish women with premalignant and cervical cancer.

Methods: 52 specimens with invasive cervical cancer (ICC) and 50 specimens with high grade cervical intraepithelial neoplasia (CIN 2/3) were identified. HPV genotyping in paraffin-embedded specimens was performed by deparaffinization of the tissue sections and DNA extraction, followed by HPV genotype detection. Quality of the extracted DNA was tested using RNAse P real-time PCR assay. HPV genotyping was carried out by using a validated commercially available PCR based HPV GenoArray test kit (Hybribio Limited, Hong Kong), which makes use of both DNA amplification and HybriBio's proprietary flowthrough hybridization technique to simultaneously identify 21 HPV genotypes.

Results: The mean age of the ICC patients was 49.2 years. Forty eight (48/52 92.3%) cervical cancer samples demonstrated PCR amplifiable DNA. 40/48 (83.3%) of the samples were HR HPV positive. HPV16 and 18 dominated covering 28/48 (58.3%) and 14/48 (29.16%) of the samples. HPV16 and 18 co-infected all six cases of multiple HR HPV infection. HPV68, 45 and 56 followed thereafter in prevalence. When considering all infections with HPV types (HPV16, 18, 31, 33, 45 and 52) that potentially may be covered by the present HPV vaccines, they accounted for 40/48 (83.3%) of the cases.

In CIN 2/3 samples, 37/47 (78.7%) samples demonstrated PCR amplifiable DNA and 20/37 (54.0%) of these samples were infected by HPV. HPV16 was found in 19/20 (95.0%) of the cases. HPV18 was found in 3/20 (15.0%) of the cases, hence HPV16 and 18 contributed to 100% of the cases.

Conclusion: HPV16 and 18 were responsible for 81.2% of the ICC specimens and 100% of the CIN2/3 specimens. Therefore it is essential to include the HPV vaccine in the vaccine schedule of the Israeli population.

O-075

microRNA-Mediated Overexpression of IQGAP3, a Novel cdc42 Binding Protein, Drives the Proliferation of Uterine Leiomyosarcoma. WeiWei Shan, Matthew L Anderson. *Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX, USA.*

Uterine leiomyosarcoma (ULMS) is a rare but highly aggressive cancer that arises in uterine smooth muscle. Very little is currently known about the molecular events involved in its pathogenesis. Using cDNA microarray analysis, we discovered that IQ motif-containing GTPase activating protein 3 (IQGAP3), a novel Rac1 and Cdc42 binding protein is over-expressed 29-fold ($p<0.001$) when specimens of ULMS (n=8) were compared to normal myometrium (n=8) or benign leiomyomas (n=8). We confirmed that IQGAP3 mRNA and protein are both markedly elevated in ULMS by Western blot and quantitative real-time PCR. Although levels of IQGAP3 in healthy myometrium were close to undetectable, primary cultures established from myometrial specimens (n=5) exhibited a considerable increase in IQGAP3 expression. Moreover, expression of IQGAP3 steadily increased as primary myometrial cultures were serially

passaged, suggesting that this gene product plays a role in cell adhesion and myometrial adaptation to culture. A marked arrest in proliferation was observed when expression of IQGAP3 was ablated in LEIO505, an established leiomyosarcoma cell line. Additionally, ectopic expression of IQGAP3 in an immortalized myometrial cell line, DD, augmented proliferation (n=3, $p< 0.05$). To determine potential causes for altered IQGAP3 expression, we used established bioinformatic platforms (Targetscan) to identify human microRNAs potentially targeting IQGAP3. We have previously found that the expression of miR-140-3p is drastically reduced in ULMS. In addition, we have now found that the predicted mRNA for IQGAP3 contains a potential binding site for miR-140-3p in its 3'-UTR. These findings suggest that the decreased levels of miR-140-3p in ULMS contribute to IQGAP3 over expression. Consistent with this hypothesis, transfecting ULMS cell lines with mimics for miR-140-3p significantly inhibited IQGAP3 expression when compared to controls.
Collectively, these observations indicate that IQGAP3 plays a key role in regulating the growth and attachment of uterine smooth muscle. A novel pathway involving miR-140-3p appears to drive increased IQGAP3 levels in ULMS. Future work will focus on elucidating the nature of this novel microRNA-mediated pathway and determining how our observations can be used to improve the diagnosis and treatment of ULMS.

O-076

Activating the Wnt/β-Catenin Pathway Does Not Initiate Endometrial Carcinogenesis, but Rather Propagates the Disease. Marten van der Zee,[1] Yundan Jai,[1] Yongyi Wang,[1] Claudia Heijmans-Antonisen,[1] Patricia Ewing,[2] Patrick Franken,[2] Francesco DeMayo,[3] John Lydon,[3] Curt Burger,[1] Riccardi Fodde,[2] Leen Blok.[1] *[1]Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, Zuid Holland, Netherlands; [2]Department of Experimental Pathology, Erasmus MC University Medical Center, Rotterdam, Zuid Holland, Netherlands; [3]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.*
Endometrial cancer arises through a gradual series of histological changes, each of which is accompanied by specific alteration in gene expression and activity. Activation of the Wnt/β-catenin pathway and loss of PTEN activity are observed in endometrial cancers. However, the role of Wnt/β-catenin and PTEN/AKT signaling in the etiology and/or progression of endometrial cancer is still unclear. Here, we investigate the effect of Wnt/β-catenin activation combined with or without Pten activity. To address this aim, mice with specific inactivation of the *Apc* gene and/or *Pten* gene in the uterus were generated and analyzed at 20, 40 and 60 weeks of age. These analyses revealed that loss of *Apc* function in the endometrium leads to activated β-catenin signaling, hyperplasia, and squamous cell metaplasia (as indicated by increased P63 and CK14 expression). However, in time the P63 and CK14 expressing cells in *Apc* mutant mice displayed a reduced in nuclear β-catenin accumulation, and increased Pten expression. When the Wnt/β-catenin pathway was activated in combination with one mutant Pten allele, an accelerated the loss of the second *Pten* allele was detected by PCR, resulting in endometrial cancer. However, loss of *Pten* function did not accelerate the loss of the second *Apc* allele In conclusion, activation of the Wnt/β-catenin pathway is associated with endometrial hyperplasia and squamous cell metaplasia that, however, does not proceed to endometrial cancer. The absence of tumor initiation in *Apc* mutant mice could be related to the increase in Pten expression in SCM, because *Pten* overexpression suppresses tumor cell growth. Activation of the Wnt/β-catenin pathway does propagate endometrial carcinogenesis by influencing chromosomal instability.

O-077

***Chlamydia trachomatis* Induces a Trophoblast IL-1β Response through the Nod-Like Receptor, Nod1, and Not through the Nalp3/ASC Inflammasome.** Vikki M Abrahams,[1] Crina Boeras,[2] Melissa J Mulla,[1] Paula B Kavathas.[2] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University; [2]Department of Laboratory Medicine and Immunobiology, Yale University.*
Objective: Bacterial infections threaten pregnancy outcome and fetal well-being by gaining access to the placenta. While the intracellular bacterium, *Chlamydia trachomatis* (Ct), can infect the placenta and modulate trophoblast function, little is known about the mechanisms involved. We previously demonstrated that Ct infection induces human first trimester trophoblast to secrete high levels of IL-1β. IL-1β secretion occurs after pro-IL-1β is processed into its active form, and we recently demonstrated that in the trophoblast, this can be mediated by an inflammasome composed of the Nod-like receptor (NLR), Nalp3, apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1. Therefore, the objective of this study was to determine if Ct infection induces trophoblast IL-1β secretion via the Nalp3/ASC inflammasome.
Methods: The human first trimester trophoblast cell lines, Sw.71 and HTR8, were infected with or without Ct (Serovar D; MOI=1) for 12-36 hrs after which RNA, protein and culture supernatants were collected. The secretion of IL-1β was measured by ELISA and multiplex analysis. IL-1β processing and caspase-1 activation was determined by Western blot. Nod1, Nalp3, and ASC knockdown in Sw.71 cells was achieved using lentiviral transfection of specific shRNAs, with a scramble shRNA serving as a control.
Results: Infection of trophoblast cells with Ct significantly upregulated IL-1β mRNA expression, increased both pro-IL-1β and active-IL-1β protein expression, and upregulated IL-1β secretion. This correlated with an increase in active caspase-1 expression (n=3; $p<0.05$). Knockdown of the inflammasome components, Nalp3 and ASC, had no effect on the ability of Ct to induce trophoblast IL-1β secretion. However, knockdown of Nod1 expression significantly inhibited the ability of Ct infection to induce trophoblast secretion of IL-1β by 68.0±17.9% when compared to the scramble shRNA control (n=4; $p<0.05$).
Conclusion: This study demonstrates that IL-1β production following first trimester trophoblast infection with Ct is independent of the Nalp3/ASC inflammasome, and is instead mediated by the non-inflammasome NLR, Nod1. This represents a novel mechanism by which certain bacterial infections induce an IL-1β response by the placenta and may, therefore, impact pregnancy outcome.

O-078

Congenital CMV Infection: Limits of Viral Load and Prenatal Imaging in Predicting Infection and Disease during Mid and Late Pregnancy. Lorinne Levitt,[1] Dan V Valsky,[1] Nili Yanai,[1] Drorith Hochner-Celnikier,[1] Dana Wolf,[2] Simcha Yagel.[1] *[1]Division of Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [2]Clinical Microbiology & Infectious Diseases, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*
Introduction
Assessing congenital CMV infection and disease is an issue of concern with limited tools. In our study we evaluated prediction of real-time PCR for infection and viral load, and prenatal imaging for infection and disease in mid and late pregnancy.
Materials & Methods
Amniotic fluid (AF) specimens obtained from women with suspected infection between 2003-2010 were tested by quantitative real-time PCR for the presence of CMV and viral load. Maternal infection was categorized as primary or non-primary according to serologic data. Prenatal ultrasound scans were performed. Infection and disease were determined by histopathlogic examination of fetus and placenta, and evaluation of the newborn.
Results
862 AF specimens were included. Maternal infection was categorized as primary in 32%. CMV was detected in 55 cases, all from women with primary infection. Real-time PCR had 100% specificity and 95% sensitivity for infection. Analysis of AF viral load revealed a large range of overlap in pregnancies with and without disease, and did not correlate significantly with US findings. Median AF viral load was significantly higher for fetuses with disease ($P=0.04$). The specificity and PPV of viral load threshold $\geq 10^5$ copies/ml were 40% and 57.1%, respectively.
Of positive cases, 18 delivered, 31 terminated, and 6 were lost to follow-up. Typical abnormal ultrasound findings were detected in 9/55. Fetal MRI was abnormal in 5/7 conducted. Disease was observed in 11/31 terminations, and 6/18 newborns. In 3/6 newborns with disease, prenatal imaging was normal. Early ultrasound was not predictive of fetal infection or disease.
Conclusions
All positive cases occurred in mothers with primary infection. Real-time PCR is reliable for assessing fetal infection. Low PPV and specificity of viral load threshold preclude reliable prenatal disease prediction. Mid-pregnancy ultrasound was not reliable for predicting fetal infection. Normal imaging in late pregnancy did not exclude disease in newborns, especially the late sequelae of congenital CMV. A protocol that combines repeated ultrasound and fetal MRI may be promising, but small numbers of newborns in this study limit this conclusion: larger studies are required.

O-079

S-nitrosoglutathione Reductase (GSNOR) Plays an Important Role in Promoting Fetal Growth and Survival and Maternal Cardiovascular Adaptation to Pregnancy in Mice. Shathiyah Kulandavelu,[1,2] Joshua M Hare.[1,2] [1]*Interdisciplinary Stem Cell Institute, University of Miami;* [2]*Department of Medicine, University of Miami Miller School of Medicine, Miami, FL, USA.*

Introduction: Preeclampsia is a condition associated with increased levels of S-nitrosylated proteins, maternal endothelial dysfunction and decreased fetal growth and survival. Increased levels of S-nitrosylated proteins are found in mice lacking S-nitrosoglutathione reductase (GSNOR), a denitrosylase that regulates S-nitrosylation. We hypothesized that GSNOR knockout (KO) mice exhibit decreased fetal growth and survival in part due to abnormal maternal cardiovascular adaptation to pregnancy.

Methods: GSNOR KO (n=7) and control C57Bl/6J mice (n=8) were studied prior to and during late pregnancy (17.5 d) while under light isoflurane anesthesia. We measured left ventricular (LV) chamber dimensions and wall thicknesses and calculated cardiac output (CO), stroke volume (SV), and relative wall thickness (RWT) using high-resolution micro-ultrasound.

Results: GSNOR KO mice had fewer (4.7 ± 1 vs. 8.1 ± 0.5 in controls, P<0.01) and smaller (0.74 ± 0.02 g vs. 0.83 ± 0.02 g in controls, P<0.01) pups. Poor fetal outcome was associated with impaired maternal cardiovascular adaptation. The normal increase in maternal cardiac output (12 ± 6% vs. 57 ± 9% in controls, P<0.001) and stroke volume (12 ± 6 vs. 44 ± 8% in controls, P<0.01) were blunted in pregnant GSNOR KO mice during pregnancy. Furthermore, at late gestation, LV end-diastolic dimension was lower (3.52 ± 0.12 mm vs. 3.78 ± 0.04 mm in controls, P<0.05), whereas anterior wall thickness was higher (1.08 ± 0.07 mm vs. 0.91 ± 0.03 mm in controls, P<0.05), contributing to higher RWT (0.56 ± 0.04 vs. 0.41 ± 0.01 in controls, P<0.01) in GSNOR KO mothers as compared to controls, consistent with concentric hypertrophy. In addition, maternal heart weight (10%, P<0.01) and kidney weights (18%, P<0.05) were higher in pregnant GSNOR KO mice as compared to controls.

Conclusion: These results suggest that regulation of S-nitrosylation by GSNOR plays an important role in promoting fetal growth and survival in part by enhancing maternal cardiovascular adaptation to pregnancy. As preeclampsia is associated with dysregulation of protein S-nitrosylation, these present findings have major implications for understanding the pathogenesis of this disease.

O-080

Increased Maternal Cortisol Increases Uterine Blood Flow and Alters Glucose Homeostasis. Maureen Keller-Wood, Xiaodi Feng. *Dept of Pharmacodynamics, University of Florida, Gainesville, FL, USA.*

Cushing's Disease in pregnancy is associated with increased maternal and fetal morbidities, however the mechanisms of cortisol effects on maternal and fetal health are not fully understood. To determine the effect of chronic increases in cortisol on maternal physiology in late gestation, pregnant ewes were instrumented at 115 days gestation with catheters and a flow probe on the main uterine artery of the pregnant uterine horn. Ewes were studied from 120-140 days (n=8 control and 6 cortisol-infused). Ewes were continuously infused with 1 mg/kg/d cortisol; on days 120, 125, 130, 135 and 140 blood was collected for measurement of plasma cortisol, insulin and glucose, and uterine blood flow was measured over a 30 minute period; all measurements were performed under basal, unstressed conditions. Infusion of cortisol chronically increased maternal cortisol to levels normally observed during mild stress (control ewes: 7.7±0.8 ng/ml, cortisol-infused ewes: 14.3±0.9 ng/ml). Uterine blood flow, measured as either as absolute flow or % flow relative to 120 days, was significantly greater in the ewes infused with cortisol (mean flow in control ewes: 412±28 ml/min or 103±4%, cortisol-infused ewes: 523±31 ml/min or 119±5%; p<0.05 for group effect in repeated measures 2way ANOVA). Plasma glucose concentration was also significantly higher in the mothers infused with cortisol from 120-140 days (mean glucose values control: 59.4±1.9 vs cortisol: 70.2±2.1 mg%); there was no difference in maternal insulin concentration (control: 1.05±0.14 ng/ml, cortisol:1.45±0.16 ng/ml). In response to glucose challenge (0.4 g/kg iv), mean insulin concentrations from 2-30 minutes and the calculated initial phase insulin response was lower in the cortisol-infused ewes than in the control ewes (AUC 2-20 min, control: 60.9±7.6, cortisol: 37.0±7.6 ng•min•ml^{-1}). The mean glucose concentrations at 60-180 minutes were higher, and the rate of decay of plasma glucose was significantly slower, in the cortisol-infused ewes (glucose=a*exp(-*t)+c*exp(-d*t; d in controls: 0.021±0.001, cortisol: 0.016±0.001 sec^{-1}). These results suggest that chronic increases in maternal cortisol produce maternal hyperglycemia and increased uterine blood flow, but also result in impaired insulin secretion in response to further increases in glucose. These results suggest that increased maternal cortisol or maternal stress can contribute to disruptions in maternal glucose homeostasis in late gestation pregnancy.

O-081

Postpartum Screening after Preeclampsia: Can We Identify Women at Risk for the Development of Chronic Hypertension? Julia J Spaan, Simone Sep, Veronica Lopes van Balen, Marc EA Spaanderman, Louis LH Peeters. *Obstetrics and Gynecology, Maastricht University Medical Center, Maastricht, Netherlands.*

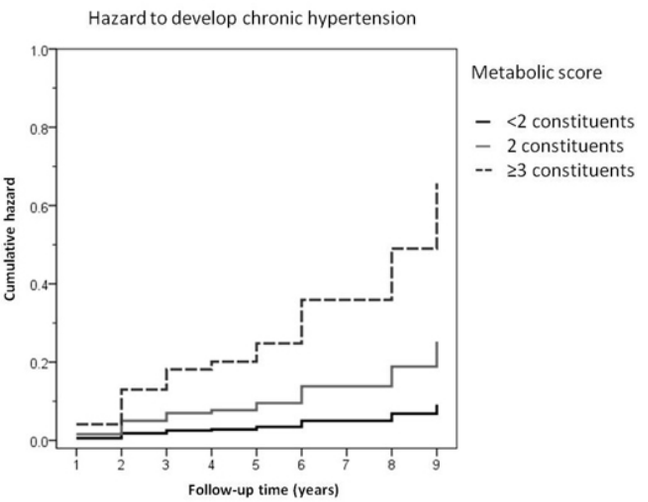
Introduction: After preeclampsia, 25% of all women will develop chronic hypertension and this may relate to the metabolic syndrome. Identification of women at risk for chronic hypertension after preeclampsia may offer opportunities for primary prevention.

Objectives: To identify risk factors for the development of chronic hypertension after preeclampsia in primiparous women who were normotensive at a 6 months postpartum screening.

Methods: Measurements at postpartum screening included assessment of BMI, automated blood pressure, microalbuminuria and fasting plasma levels of glucose, insulin and lipid profile. Women hypertensive at postpartum screening (17%) were excluded from this analysis. In the normotensive women we evaluated the development of chronic hypertension by self-reported antihypertensive treatment on a short health questionnaire. The metabolic syndrome was defined by ATP-III criteria.

Results: At a response rate of 72% during a median 6 year follow-up, 27 women out of 377 (8%) developed chronic hypertension. The hazard rate for the development of hypertension was 3.1 (95%-CI 1.3-7.4) for obesity, 3.8 (95%-CI 1.6-8.8) for high-normal blood pressure, 3.7 (95% CI 1.4-10.0) for family history of hypertension and 4.3 (95% CI 1.6-11.5) for recurrent hypertensive disease in pregnancy. Women with 2 and ≥3 constituents of the metabolic syndrome were 2.9 (95% CI 1.2-7.5) and 8.1 (95% CI 2.8-22.9) times more likely, respectively, to develop chronic hypertension.

Conclusion: Besides family history of hypertension and recurrent hypertensive disease in pregnancy, normotensive primiparous women with a history of preeclampsia are at risk for later chronic hypertension when having 2 or more constituents of the metabolic syndrome. These findings support the view that lifestyle interventions may prevent or delay the development of chronic hypertension in women with a history of preeclampsia.



O-082

Maternal Plasma Endotoxin Increases Significantly across Pregnancy with No Association with Obesity, Inflammation, or Insulin Sensitivity. Imari Lee, Nicole Kotchey, Robin E Gandley, Arun Jeyabalan, Carl A Hubel, Robert W Powers. *Magee-Womens Research Institute, Dept OBGYN-RS, University of Pittsburgh, Pittsburgh, PA, USA.*

Context: Endotoxin activates innate immunity, decreases insulin sensitivity and is associated with obesity. Recent data indicates that subclinical endotoxemia is associated with inflammation in obese women in late pregnancy.

Objective: The objective of this study was to quantify circulating endotoxin across pregnancy in lean and obese women, and assess the relationship between endotoxin and markers of inflammation and insulin sensitivity.

Study Design: Endotoxin was measured in sterile maternal EDTA plasma samples from 24 lean pregnant women (BMI=22.4±1.9 kg/m2) and 45 obese pregnant women (BMI= 32.6±2.1 kg/m2), and 6 non-pregnant women. Samples were collected at 10.5±3.1, 21.3±4.6 and 35.2±2.1 weeks gestation. Endotoxin was quantified using the PyroGene Recombinant Factor C Endotoxin Detection Assay from LONZA, inter-assay variability <10%. IL-6, myloperoxidase, uric acid, triglycerides, insulin and glucose were also measured. Statistical analysis was by repeated measures ANOVA and Students t-test as appropriate. Correlation analysis was performed using Pearson product moment correlation coefficient. Statistical significance was accepted at $p<0.05$.

Results: Endotoxin was significantly increased in both lean (10.4±5.3 EU/ml) and obese (9.1±5.3 EU/ml) pregnant women compared to non-pregnant women (4.3±2.6 EU/ml, $p<0.05$). Endotoxin increased significantly across pregnancy in both lean and obese pregnant women ($p<0.001$), but was not different between these groups (table). Endotoxin was not associated with adiposity, IL-6, myloperoxidase, uric acid, triglycerides or insulin sensitivity as assessed by homeostasis model of insulin resistance (HOMA).

Conclusion: Circulating endotoxin increases significantly during pregnancy, but endotoxin is not associated with markers of systemic inflammation or insulin resistance. Pregnancy may represent a condition of metabolic endotoxemia, however the causes and biologic activity of these increasing levels of endotoxin are unclear.

Endotoxin (EU/ml)	First trimester (10.5±3.1 weeks)	Second trimester (21.3±4.6 weeks)	Third trimester (35.2±2.1 weeks)
Lean pregnant (n=24)	10.4±5.3	15.5±8.8	16.6±5.9
Obese pregnant (n=45)	9.1±5.3	15.2±9.2	18.3±11.5

Data are mean±SD. Repeated measures ANOVA $p<0.001$.

This project supported by National Institutes of Health grants P01-HD30367.

O-083

Adverse Impact of Progestin Exposure and Endometrial Shedding Prior to Ovulation Induction on Conception and Live Birth in Women with Polycystic Ovary Syndrome. Michael P Diamond, Michael Kruger, Nanette Santoro, Heping Zhang, Peter Casson, William Schlaff, Christos Coutifaris, Robert Brzyski, Gregory Christman, Bruce R Carr, Peter G McGovern, Nicholas A Cataldo, Michael P Steinkampf, Gabriella G Gosman, John E Nestler, Sandra Carson, Evan E Myers, Esther Eisenberg, Richard S Legro. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*

Objective: To determine whether progestin induced endometrial shedding, prior to ovulation induction with clomiphene citrate, metformin or a combination of both, affects ovulation, conception, and live birth rates in women with polycystic ovary syndrome (PCOS), from a secondary analysis of the data from the NICHD Cooperative Reproductive Medicine Network trial randomized, double blind, multi-center clinical trial (PPCOS I).

Methods: 626 PCOS women randomized to up to six cycles of clomiphene citrate alone, metformin alone, or clomiphene citrate plus metformin were assessed for occurrence of ovulation, conception, and live birth associated with prior menses (either due to ovulation or exogenous progestin induced withdrawal bleed), as well as the relation to androgenic and metabolic milieus.

Results: While ovulation rates were higher in cycles preceded by spontaneous endometrial shedding than after anovulatory cycles (with or without prior progestin withdrawal), both conception and live birth rates were significantly higher following anovulatory cycles without progestin-induced withdrawal bleeding (Live birth/cycle: spontaneous menses 2.2%, Anovulatory with progestin withdrawal 1.6%, Anovulatory without progestin withdrawal 5.3%, each $p<0.001$). The difference was more marked when the live birth rate was calculated per ovulation: Live birth/ovulation: spontaneous menses 3.0%, Anovulatory with progestin withdrawal 5.4%, Anovulatory without progestin withdrawal 19.7%, each $P < .001$.

Conclusion: Conception and live birth rates are lower in PCOS women following a spontaneous menses or progestin induced withdrawal bleeding as compared to anovulatory cycles without progestin withdrawal. These findings suggest that the common clinical practice of progestin induced endometrial shedding prior to ovarian stimulation may have an adverse impact on rates of conception and live birth in anovulatory women with PCOS.

O-084

1H-NMR Based Metabonomics of Spent Culture Media Cannot Distinguish Implantable from Non-Implantable Day 3 Human Embryos. Paolo Rinaudo,[1] Jia Hua,[2] Su Qian,[3] Uday Prabhu,[2] Erwin Garcia,[2] Marcelle Cedars,[1] Danish Sukumaran,[2] Christopher Andrews,[3] Thomas Szyperski,[1] Shehua Shen.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, UCSF; [2]Chemistry, State University of New York at Buffalo; [3]Chemistry and Biostatistics, State University of New York at Buffalo.*

Objectives: To predict success of embryo implantation based on 1H NMR profiles of spent culture media and to identify variations in media component concentrations that correlate with embryo implantation.

Design: Retrospective study.

Materials and Methods: Individual spent media samples (14 µL) from embryos that implanted (n=123) and samples from embryos that failed to implant (n=156) were individually collected on day 3 and evaluated using 1H NMR. Different methods of resonance assignments were utilized including NOESY/CPMG and 2D-J/CPMG.

Target profiling was performed in a subset of samples. Data obtained with the skyline projection of the 2DJ experiments underwent Fourier Transformation and base-line correction; spectra were then bucketed into 1,600 equal-width bins and normalized to unit sum. The buckets were then mean-centered and Pareto-scaled. Subsequently, logistic regression of implantation status on the principal components of the buckets was performed. The analysis was cross-validated to provide accurate assessment of predictive ability. Additionally, univariate analysis of each bucket was used to identify biomarkers of successful implantation. Data processing and statistical analyses were completed in the R and MATLAB computing environments.

Results: High-quality NMR spectra were obtained for all media samples. Separation of implantable and non-implantable embryos was minimal and did not allow building of a predictive model. Putative surrogate biomarkers of implantation suggested by others were not validated.

Conclusion: We found no correlation between profiles of spent culture media and implantation potential. Although the effects of heterogeneity of patients and of media composition were ameliorated by control media samples, the metabolic signatures of successful and unsuccessful embryos are too similar for accurate prediction.

Support: NICHD-R21: HD054956-01A2

O-085

Flutamide Treatment Reverses Endothelial Dysfunction in a Rat Model of Polycystic Ovarian Syndrome (PCOS). Jennifer Keller,[1] Amanda Hurliman,[1] Maurizio Mandala,[1,2] Peter Casson,[1] George Osol.[1] *[1]Reproductive Endocrinology, Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Cell Biology, University of Calabria, Cosenza, Italy.*

Methods: Female rats were randomized at 3-4 weeks to implantation of a 7.5 mg, 90-day dihydrotestosterone (DHT) pellet or a matched placebo as previously described in our lab, utilizing a novel rat model exhibiting both metabolic and ovarian aspects of PCOS (model: Manneras et al, 2007). After exposure to DHT for 8-9 weeks through puberty and into early adulthood, six rats were treated with a 60mg, 30-day flutamide pellets beginning at 11 weeks of age. At 15 weeks, all rats were weighed, blood pressure was measured through a tail cuff, and animals were decapitated. Experiments were then performed on isolated mesenteric arteries using a pressurized arteriograph. Endothelial function was assessed by the response (efficacy) of pre-constricted arteries to acetylcholine (ACh). Differences between groups were determined by ANOVA.

Comparison of Control, DHT, and Flutamide-treated DHT rats

	Control	DHT	Flutamide-treated
Weight (g)	232 ± 6.0	299 ± 6.9[1]	307± 7.0[2]
Blood Pressure (mmHg)	122/85	137/98[1]	118/79[3]
Mesenteric artery diameter at 50mmHg	227± 8	212 ± 4	220 ± 8
Efficacy (% maximal dilation)	100 ± .1	78 ± 6[1]	99.3 ± .7[3]

[1]$p<0.05$ for PCOS vs. Control; [2]$p<0.05$ Flutamide-treated PCOS vs. Control; [3]PCOS vs. Flutamide-treated PCOS

Results: Flutamide-treatment of DHT rats from 11-15 weeks normalized blood pressure; however, had no effect on adult rat weight. Further, whereas untreated DHT rats demonstrated significantly reduced efficacy to ACh compared to controls, treatment of adult DHT-rats with flutamide restored normal vasodilatory efficacy. **Conclusion:** Clinically, PCOS is associated with endothelial dysfunction, a pathologic state widely believed to be a hallmark of vascular disease. We have previously demonstrated the presence of endothelial dysfunction in a hyperandrogenic rat model of PCOS. Here we show reversal of this dysfunction with the use of anti-androgen treatment,

even after establishment of disease and secondary effects (e.g. weight gain) during puberty. This data provides insight allowing for more targeted research regarding the androgenic contribution to endothelial dysfunction in PCOS and potential therapeutic interventions.

O-086

Significance of Moderately Abnormal Basal FSH and/or Estradiol-17β in Subfertile Women Undergoing Complete Justifiable Treatment with IUI or IVF: Results of FASTT and FORT-T. DJ Kaser,[1] MB Goldman,[2] JL Fung,[2] MM Alper,[3] RH Reindollar.[2] *[1]Dept OB-GYN, Brigham & Women's Hospital, Boston, MA; [2]Dept OB-GYN, Dartmouth-Hitchcock Medical Center, Lebanon, NH; [3]Dept OB-GYN, Boston IVF, Waltham, MA.*

Objective: To determine if moderately abnormal FSH and/or estradiol-17β (E_2) levels affect pregnancy outcomes in subfertile women undergoing IUI or IVF with mandated insurance coverage

Design: Secondary analysis of two prospective randomized trials

Methods: Cycle demographics, variables and outcomes were pooled from the Fast Track and Standard Treatment Trial (FASTT) and Forty and Over Infertility Treatment Trial (FORT-T). FASTT (n=503) randomized women ages 21-39 to traditional or accelerated treatment with clomiphene citrate IUI (CC-IUI), gonadotropin IUI (FSH-IUI), and IVF; FORT-T (n=154) randomized women ages 38-43 to superovulation IUI (SO-IUI: CC or FSH) then IVF or immediate IVF. Patients were treated until they no longer demonstrated a reasonable chance for success. Four groups were identified according to day 3 FSH and E_2 values (Table 1). Live birth rates were calculated for each group and treatment modality. Data were analyzed by ANOVA for continuous variables and Fisher's exact for categorical variables.

Results: Patients with FSH ≥ 10 were older than those with FSH < 10 (p=0.01). No live births occurred in Group 2B during SO-IUI (0/21) (p=0.02). When age was examined, no live births (0/28) occurred during SO-IUI among women ≥ 40 years with FSH ≥ 10 (n=19), regardless of E_2 concentration.

Table 1. Cycle outcomes stratified by FSH and E_2 levels

	Group 1A	**Group 1B**	**Group 2A**	**Group 2B**	
	FSH < 10 E_2 < 50 (n=406)	FSH < 10 E_2 ≥ 50 (n=141)	FSH ≥ 10 E_2 < 50 (n=48)	FSH ≥ 10 E_2 ≥ 50 (n=8)	*P* value
Age (y)	34.6 ± 4.2	34.4 ± 4.3	36.4 ± 4.3	36.6 ± 3.9	0.01
Day 3 FSH (mIU/mL)	6.5 ± 1.6	6.3 ± 2.0	11.7 ± 1.4	12.1 ± 1.6	<0.001
Day 3 E2 (pg/mL)	34.1 ± 8.4	64.5 ± 12.5	34.4 ± 7.5	66.7 ± 13.0	<0.001
CC-IUI Live Birth Rate	72/885 (8.1)	12/337 (3.5)	6/81 (7.4)	0/17 (0)	0.03
FSH-IUI Live Birth Rate	30/319 (9.4)	8/139 (5.8)	2/45 (4.4)	0/4 (0)	NS
IVF Live Birth Rate	132/594 (22.2)	52/202 (25.7)	9/79 (11.4)	2/15 (13.3)	NS

Mean ± SD or n (%)

Discussion: Patients with FSH ≥ 10 and E_2 ≥ 50 are unlikely to achieve live birth with CC-IUI or FSH-IUI, even if afforded complete treatment. In women ≥ 40 years with FSH ≥ 10, SO-IUI may not be an effective treatment option. This provides further support for beginning therapy with immediate IVF in women of advanced reproductive age.

O-087

Limbic Activation with Emotional Processing Associated with Central Opioid Activity in Polycystic Ovary Syndrome. Courtney A Marsh, Alison Berent-Spillson, Tiffany Love, Rodica Pop-Busui, Carol C Persad, Jon-Kar Zubieta, Yolanda R Smith. *Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA.*

Objective: Polycystic Ovary Syndrome (PCOS), both a reproductive and metabolic disorder, is associated with increased risk of insulin resistance (IR) and mood disorders. Although the etiology of mood disorders is unclear, recent studies suggest involvement of the opioid and glucose regulatory systems. Our goal was to assess whether changes seen in limbic activation with metformin therapy are associated with changes in the mu-opioid system.

Design: Matched case-control pilot study in an academic university setting.

Materials and Methods: Participants included seven IR-PCOS women and five non-insulin resistant controls, aged 21 – 40 years. We assessed metabolic function, mood, neuronal activation during an emotional task using functional magnetic resonance imaging (fMRI), and mu opioid receptor availability using [^{11}C] carfentanil positive emission tomography (PET). In IR-PCOS women, assessments were repeated after 16 weeks of metformin (1500 mg/day). During fMRI, subjects rated emotional pictures as unpleasant or neutral. Mu-opioid receptor availability (non-displaceable binding potential) was calculated from PET images using a modified Logan analysis with occipital cortex as reference region. Image data were analyzed using SPM2 and SPSS. Clinical and metabolic measures were compared using Mann-Whitney (IR-PCOS versus controls). Associations between changes in regional synaptic activity and mu-opioid receptor availability pre- and post- metformin were assessed using Pearson correlations.

Results: IR-PCOS women had higher waist circumference, BMI, free testosterone, fasting insulin, and HOMA-IR compared to controls (p≤0.01). A decrease in mu-opioid receptor availability in the amygdala and nucleus accumbens associated with metformin therapy correlated with greater activation in the right ventral anterior cingulate and nucleus accumbens (p≤0.05) during the emotion task.

Conclusions: After metformin therapy, there was an inverse relationship between fMRI activation during emotional processing and mu opioid receptor availability in areas of the brain related to emotional processing, motivation, and memory of emotional reactions. This suggests that availability of mu opioid receptors may affect emotional processing in IR-PCOS women and may be modifiable with metformin therapy.

O-088

Live Birth and Clinical Pregnancy Rates after Elective Single Embryo Blastocyst Transfer Are Strongly Predicted by Age and Trophectoderm Score. Ndidiamaka Onwubalili,[1] Kelecia Brown,[1] Stephanie M Thompson,[1] Sangita K Jindal,[2] Peter G McGovern.[1] *[1]Obstetrics, Gynecology and Womens Health, New Jersey Medical School, Newark, NJ, USA; [2]Montefiore's Institute for Reproductive Medicine and Health, Albert Einstein College of Medicine, Hartsdale, NY, USA.*

Recent advances in in-vitro fertilization (IVF) have found that blastocyst stage embryos result in higher live birth and implantation rates as compared to cleavage stage embryos. Although IVF continues to yield excellent pregnancy rates with blastocyst transfer, clinical pregnancy (CP) rates from elective single embryo transfers (eSET) are generally not comparable with pregnancy rates from transfer of multiple embryos. We hypothesized that characteristics of blastocyst morphology such as the trophectoderm (TE) score, inner cell mass score (ICM), embryo grade and stage would serve as significant predictors of both live birth (LB) and clinical pregnancy rates, as analyzed using an eSET model from a large cohort.

Data from 3151 cycles of fresh, non-donor eSET cycles from 2008-2009 were obtained from the Society for Assisted Reproductive Technologies (SART). All eSET were performed at the blastocyst stage. There were 1526 live births (48.4%). Multiple logistic regression analysis (Sigmastat 11.0) was performed to evaluate significant independent predictors of CP and LB. Statistical significance was determined by p <0.05. Variables included in the model were patient age, trophectoderm (TE) score, inner cell mass (ICM) score, embryo stage and embryo grade. TE, ICM and embryo grade were scored as poor, fair or good. Embryo stage was described as early blast, expanded blast or hatching blast. Clinical pregnancy was significantly predicted by better TE score (p=0.003), better embryo grading (p=0.013) and lower patient age (p<0.001). LB was significantly predicted only by lower age (p<0.001) and better TE scoring (p=0.014), but not by embryo grade(p=0.132). ICM scoring was not predictive of either LB (p=0.369) or CP rates (p=0.458). Similarly, embryo stage was also not predictive of LB (p=0.177) or CP rates (p=0.150). TE scoring and patient age are significant predictors of both LB and CP after eSET. Surprisingly, ICM is not the most important predictor of LB and CP. As demonstrated in this large, multi-center analysis, selection of an embryo with a high TE score may result in improved ability to predict successful single embryo transfer.

O-089

Endometrial Stromal Cells of Women with Recurrent Miscarriages Fail To Discriminate between High- and Low-Quality Embryos. Charlotte HE Weimar,[1] Annemieke Kavelaars,[1] Jan J Brosens,[2] Johanna MT de Vreeden-Elbertse,[1] Cobi J Heijnen,[1] Nick S Macklon.[3] *[1]Laboratory of Neuroimmunology and Developmental Origins of Disease (NIDOD), University Medical Center Utrecht, Netherlands; [2]Division of Reproductive Health, Warwick Medical School, United Kingdom; [3]Division of Developmental Origins of Adult Diseases (DOHaD), University of Southampton, United Kingdom.*

Introduction

The etiology of recurrent miscarriages (RM) remains largely unexplained. Recent studies show that women with RM have a shorter time to pregnancy interval than normally fertile controls, indicating they may have a more receptive endometrium, which may facilitate implantation of abnormal

embryos. We hypothesized that human endometrial stromal cells (H-ESC) of women with RM discriminate less effectively between high- and low-quality embryos than H-ESCs from normal fertile controls. To test this hypothesis, we quantified *in vitro* directed migration of H-ESCs obtained from women with RM towards high- or low-quality human embryos with that of H-ESCs obtained from fertile controls.

Methods

We used H-ESCs from endometrial biopsies of 3 women with RM and 3 fertile age-matched controls. A cell-free strip, the migration zone, was created in confluent monolayers of decidualized H-ESCs. A high-quality (day 5 blastocyst, n=13), low-quality (day 5: 3PN blastocyst or underdeveloped embryo, n=12), or no (controls) human embryo was placed in the migration zone. The migratory response was quantified after 18 hours.

Results

H-ECS from fertile control women migrated only towards high-quality embryos and not towards low-quality embryos ($P<0.05$). In fact, migration of H-ECS from fertile controls in response to a low quality embryo was reduced compared to basal migration in the absence of an embryo ($P<0.05$). Interestingly, the migratory response of H-ESCs from women with RM did not differ between high- and low-quality embryos. Moreover, migration of H-ESCs from women with RM towards high- and low-quality embryos was similar to that of H-ESCs from fertile women towards high quality embryos. In the absence of an embryo migration of H-ESCs from fertile or RM women was similar. In addition, the proliferative activity of H-ESCs did not differ between fertile and RM women.

Conclusions

The migration of H-ESCs from fertile women is dependent on the quality of the embryo and is inhibited by low quality embryos. In contrast, H-ESCs from RM women fail to discriminate between high- and low-quality embryos.

O-090

The First Report of a SSEA1+ Human Endometrial Epithelial Cell Subtype with the Hallmarks of an Adult Stem/Progenitor Cell. DK Hapangama,[1] A Valentijn,[1] J Drury,[1] G Saretzki,[2] A Rak-Raszewska,[1] P Murray,[1] C Gargett.[3] *[1]Liverpool University, United Kingdom; [2]Newcastle University, United Kingdom; [3]Monash University, Australia.*

Objectives Epithelial stem/progenitor cell activity has been identified in the highly regenerative human endometrium. There are currently no specific markers for endometrial epithelial stem/progenitor cells (EEpSPC) and their culture is challenging. We aimed to screen human endometrium for reactivity to known stem cell markers and to investigate whether SSEA1, an early differentiation marker of human embryonic stem cells isolates candidate EEpSPCs with telomerase activity and ability to differentiate into glands.

Method Pre/post menopausal human endometrial full thickness samples were screened to identify putative EEpSPC markers by IHC using a panel of embryonic and adult stem cell markers. SSEA1+ cells were analysed by FACS and isolated by magnetic cell sorting (MACS). Cells grown in 2D and 3D culture were subsequently phenotyped by immunfluorescence, immunhistochemistry, live cell imaging, TRAP, qPCR. Singly dispersed endometrial epithelial cells cultured as organoids in serum-free media in a 3D matrix were similarly characterised with IF, IHC, TRAP and qPCR.

Results SSEA1 expression was largely confined to the basal epithelial glandular cells of all endometrial tissue sections studied (n=40) with most intense staining seen in the postmenopausal (n=10) and proliferative phase (n=10). The expression of SSEA1 in 2D culture rapidly expanded from 10% to >50% following 7 days in culture. These cells were highly proliferative and also had high levels of telomerase activity. MACS sorted epithelial SSEA1+ cells seeded into 3D culture in serum-free media produced gland-like organoid structures at a much higher frequency than the SSEA1-CD9+ cells. Stromal cells did not produce organoids. The organoid-derived SSEA1+CD9+ cells also expressed CD133, CD49f, Podocalyxin, ALDH1, Nanog, hTERT, ERb, PR but not ERa.

Conclusion Here is the first report of a surface marker that defines a subset of epithelial cells with the hallmarks of a progenitor with the ability to produce endometrial gland-like structures in vitro. We propose that SSEA1+ epithelial cells are involved in the regeneration of endometrial glands following menstruation. The challenge is to further refine the signature characterising this population. Our novel 3D-culture system provides a reproducible and accessible means to investigate EEpSPC function in vitro.

O-091

Cross-Talk between Epithelial and Stromal Cells: Evidence of Paracrine Effects Using a Novel Human Endometrial Three-Dimensional (3D) Culture System. Hai Wang,[1] Silvina Bocca,[1] Sandra Anderson,[1] Jose Horcajadas,[2] Sergio Oehninger.[1] *[1]Dept of OB/GYN, The Jones Institute for Reprod Med/EVMS; [2]Araid at I+CS, Zaragosa, Spain.*

Objectives: Sex steroids are essential for developing an endometrium supportive of embryo implantation. Cells cultured in a 3D environment have been shown to better represent *in vivo* events. Using a newly bioengineered 3D endometrial culture system (Wang et al, SGI 2011) we investigated the interaction between epithelial and stromal cells in the presence and absence of 17β estradiol (E_2) and progesterone. We hypothesized that epithelial and stromal cells reciprocally affect their behavior, proving evidence for a functional interaction between these compartmentalized cells in the 3D culture.

Methods: An endometrium-like 3D culture system was constructed with a fibrin-agarose gel matrix, with epithelial cells (Ishikawa) seeded on top, and stromal cells (HESC) residing within the 3D matrix. Cultures were treated with E_2 (10^{-8} M) for 5 days, followed by E_2 and medroxyprogesterone acetate (MPA, 10^{-6} M) for additional 7 days. Prolactin (PRL) secretion by the 3D culture system into the culture medium (a marker of decidualization) was measured by ELISA. After treatment, epithelial cells were released from the top of 3D culture system by trypsin digestion. Isolated epithelial cells and stromal cells residing in the matrix were separately subjected to RNA extraction, followed by RT-PCR for examination of gene expression levels (normalized against 18S).

Results: PRL secretion significantly increased under E_2+MPA treatment ($P< 0.05$), indicating decidualization transformation within the 3D culture system. These effects were similar to the timeline of stromal cell decidualization of HESC grown in a monolayer. An effect of epithelial cells on the decidualization of stromal cells in 3D culture system was observed, with evidence that levels of PRL mRNA were significantly higher in the 3D culture system than in stromal cell monolayers on day 7 under E_2+MPA treatment ($P=0.03$). On the other hand, the relative expression of MUC1 was significantly higher in monolayers of epithelial cells treated with the steroids ($P<0.05$) but not in the 3D cultures, pointing to regulation of epithelial cells by the stroma.

Conclusions: Using a novel 3D endometrial cell culture system we demonstrated reciprocal interactions between epithelial and stromal cells, providing further support for the validity of this model to examine endometrial paracrine effects.

O-092

Connexin 43 Is Reduced in Eutopic Endometrium of Subjects with Endometriosis. Jie Yu,[1] Anisoara Boicea,[2] Kara L Barrett,[1] Christopher O James,[1] Indrani C Bagchi,[3] Milan K Bagchi,[3] Ceana Nezhat,[2] Neil Sidell,[1] Robert N Taylor.[1] *[1]Gynecology and Obstetrics, Emory University, Atlanta, GA, USA; [2]Reproductive Surgery, Nezhat Medical Center, Atlanta, GA, USA; [3]Comparative Biosciences and Molecular and Integrative Physiology, University of Illinois, Champaign/Urbana, IL, USA.*

The causes of reduced fecundity associated with endometriosis remain controversial, but evidence indicates a detrimental effect on intrauterine (eutopic) endometrial differentiation and embryonic receptivity, including a failure of decidualization. Microdomains composed of gap junctions, which facilitate cell-cell communication, may be responsible. Pharmacological and genetic inhibition of connexin (Cx) 43 blocks morphological and biochemical decidualization. Moreover, conditional deletion of Cx43 in mouse uterus led to a striking impairment of decidual differentiation and angiogenesis, resulting in implantation arrest and pregnancy failure. The current studies were undertaken to test the hypothesis that women with endometriosis have abnormal Cx43 concentrations in eutopic endometrium. Consenting subjects were recruited prior to laparoscopic exploration and endometrial biopsies were collected. Immunohistochemistry confirmed the stromal localization of Cx43 in tissue sections. Cycle phase-matched samples from women with histologically-confirmed endometriosis (12 cases) and those without (12 controls) were subjected to Western blotting to quantify Cx43 protein levels. Actin was used as an internal control for total cellular protein. Western blot signals were quantified with Image J software and compared by Mann-Whitney U-tests. The ratio (mean±SD) of Cx43/actin was 0.16±0.12 in endometriosis cases and 0.34±0.15 in control biopsies ($Z=3.12$, $P<0.01$). Endometrial stromal cells cultured from eutopic biopsies were analyzed similarly and revealed a ratio of Cx43/actin of 0.09±0.01 in endometriosis and 0.23±0.07 in controls ($Z=3.58$, $P<0.01$). Our results indicate that eutopic endometrial Cx43 concentrations in endometriosis cases are less than half those in controls, and that the levels in situ are accurately reflected in stromal cells isolated from the biopsies. Based on prior functional studies in murine and cancer models, we postulate that reduced Cx43 and the

gap junctions they comprise contribute to abnormal stromal differentiation and invasive phenotype typical of endometriosis. Supported by Eunice Kennedy Shriver NICHD, U54 HD55787 and R01 HD55379.

O-093

Bisphenol-A Exposure In Utero Programs Adult Uterine Estrogen Responsiveness. Elisa M Jorgensen, Myles H Alderman, Michael Li, Hugh S Taylor. *OB/GYN, Yale School of Medicine.*

Introduction: Bisphenol-A (BPA) is an environmentally ubiquitous estrogen-like endocrine-disrupting compound. Exposure to BPA in utero has been linked to female reproductive disorders including endometrial hyperplasia, endometriosis, fibroids and breast cancer. Estrogens have a role in the etiology of each of these conditions. Here we hypothesized that BPA exposure in utero leads to changes in developmental programming of estrogen responsive gene expression.

Methods: 8 pregnant CD-1 mice were continuously treated with BPA (5 mg/kg/day) or vehicle via osmotic minipump on days 9-18 of gestation. 2 weeks after birth, uteri of half the female offspring were isolated, and RNA was extracted. At 6 weeks, remaining female offspring were oophorectomized, then treated with a single IP injection of 300 ng estradiol (E2) or vehicle, and the uteri were removed for RNA isolation. Total RNA was labeled and hybridized to a mouse BeadChip WG-6 expression microarray (Illumina). Genes showing statistically significant change (> 2-fold) versus control were verified using real time RT-PCR.

Results: At 2 weeks, global gene expression was remarkably similar among the control and BPA exposed groups. Of a total 45,000 genes examined, only 18 (10 upregulated and 8 downregulated) showed changes in expression of 2-fold or greater. After estrogen exposure at puberty (8 weeks), the expression profile was markedly changed. At baseline, a total of 365 genes (77 upregulated and 288 downregulated) showed altered expression in BPA-exposed offspring. With E2 treatment, expression of another 316 genes (90 up and 226 down) showed altered E2 response in BPA-treated offspring; this included several genes that were not previously regulated by estrogen (e.g. FZD10, GDF10) or that demonstrated an exaggerated response to estrogen treatment (RET, WIF1, S100A8). Expression changes of those genes with the greatest fold change were verified by real-time RT-PCR.

Conclusions: Significant changes in gene expression were observed in the uteri of mice exposed to BPA as a fetus; however, these differences became apparent only after endogenous estrogen exposure at puberty or with estrogen treatment. Gene-environment interactions driven by BPA alter the normal developmental programming of estrogen responsive gene expression in the uterus. Hyperresponsiveness to estrogens is a potential mechanism to explain the increased incidence of estrogen related disorders seen after exposure to endocrine disruptors.

O-094

The Effect of Ovarian Stimulation on Placentation and Fetal Growth. Monica A Mainigi,[1] Isaac Sasson,[1] Irina Burd,[2] Christos Coutifaris.[1] *[1]Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA; [2]Department of Gynecology and Obstetrics, Johns Hopkins University, Baltimore, MD, USA.*

Introduction: Assisted reproductive technologies (ART) utilize multiple clinical and laboratory interventions to generate a cohort of embryos capable of implantation and development. One such intervention, ovarian stimulation, results in significant changes in the hormonal environment, which persist during the peri-implantation and early placentation periods. Recent epidemiologic evidence showing lower mean birth weight after fresh versus frozen embryo transfer suggests that the treatment-induced peri-implantation maternal environment plays an important role in perinatal outcomes. In this study, using the mouse model, we examine the effect of the peri-implantation hormonal milieu on placentation and fetal growth.

Methods: Blastocysts from naturally mated mice were flushed and transferred non-surgically into the uteri of pseudopregnant recipients following either natural mating or mating following treatment with gonadotropins (with vasectomized males). On post-coital day 17.5, recipient dams were sacrificed, pups and placentas were collected and weighed and fixed for subsequent histologic analysis.

Results: The mean fetal weight of pups harvested from recipients subjected to superovulation was significantly lower than naturally mated recipients (1.37 vs. 0.99 grams, $p<0.001$). Mean placental weight was also lower in placentas harvested from superovulated recipients compared to naturally mated ones ($p<0.05$). There was no significant difference in litter size. Histologically, placentas from superovulated recipients showed attenuated branching with limited invasion of the junctional zone by the labyrinth.

Conclusion: The non-physiological peri-implantation maternal hormonal environment resulting from gonadotropin stimulation appears to have a direct effect on fetal growth possibly mediated, at least in part, through alterations in trophoblast expansion and invasion. Although the specific molecular and cellular mechanism(s) leading to these observations remain to be elucidated, this line of investigation is important in order to understand the pathophysiology of adverse perinatal outcomes following assisted reproduction.

O-095

Human Endometrium Undergoes Epigenetic Modification during the Menstrual Cycle with an Altered Pattern in Women with Endometriosis. Sahar Houshdaran, Zara Zelenko, Juan C Irwin, Linda C Giudice. *Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA.*

Background: Endometriosis affects 6-10% of women of reproductive age and is characterized by endometrial tissue outside the uterus. It is an estrogen-dependent, progesterone (P4)-resistant disorder of unknown etiology. DNA methylation at the 5' position of cytosine is a main epigenetic mechanism that is essential in many biological processes such as genomic imprinting, chromosomal stability, and cancer. Our transcriptome and preliminary epigenetic studies have shown differences in the eutopic endometrium of women with and without disease. However, epigenetic modifications in normal cycling endometrium remain largely unknown and thus the extent of epigenetic abnormalities in endometriosis. Herein, we investigated the global DNA methylome of eutopic endometrium from women with and without endometriosis in different phases of the menstrual cycle.

Methods: Eutopic endometrial tissues were obtained after informed consent through the NIH UCSF Endometrial Tissue and DNA Bank, 18 from women with severe endometriosis and 18 without disease, comprising 6 samples in each of the proliferative (PE), early secretory (ESE) and mid-secretory (MSE) phases. We utilized Illumina Infinium methylation bead array that assays 27,578 CpG sites in >14,000 genes in the human genome.

Results: We expanded our preliminary epigenetic analysis of 12 samples to 36 samples. Statistical comparison among cycle phases (PE, ESE, MSE) within disease or control groups showed the highest number of differentially methylated probes to be in PE vs. MSE in no disease, but between ESE vs. MSE in disease. Comparing the epigenetic signature of disease vs. no disease by phase showed the greatest epigenetic differences were in the secretory phase with fewer differences in the proliferative phase. Since the secretory phase coincides with P4 action on the endometrium, epigenetic abnormalities may be at the root cause of P4-resistance observed in endometriosis.

Conclusion: Our expanded analysis confirms our earlier data of abnormalities in DNA methylation profiles of cycle phases in disease compared to no disease and their potential role in aberrant hormone-response in endometriosis. Differential DNA methylome across the cycle in normal endometrium suggest a crucial role for epigenetic mechanisms involved in differentiation of normal cycling endometrium and its hormonal regulation.

Support: NIH U54HD055764-05 (LCG)

O-096

Role of Stem Cells in Human Uterine Leiomyoma Growth. Masanori Ono, Wenan Qiang, Vanida Ann Serna, John V Coon, Antonia Navarro, Ping Yin, Toshiyuki Kakinuma, Stacy Druschitz, Kenji Unno, Takeshi Kurita, Serdar Bulun. *Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Uterine leiomyoma is thought to be a monoclonal tumor arising from a myometrial cell. To elucidate the cell type responsible for its growth, we explored the role of leiomyomas' side population component enriched with stem/reservoir cells in its tumorigenesis. Human uterine leiomyoma (n=42) and myometrial (n=28) tissues from hysterectomy specimens (n=53) were sorted to side or main populations by flow cytometry. Monolayer cultures of side or main population cells were either maintained alone, co-cultured in two separate compartments or in direct contact with each other. Side or main populations in mixed co-cultures were identified after initially dye-labeling each cell type. Mixtures of two cell types were also maintained in vivo as xenografts under the kidney capsule of immunodeficient mice. RESULTS: Side population cells comprised approximately 1% of leiomyoma, and 2% of myometrial cells. Leiomyoma side population cells alone did not grow in culture in the presence or absence of estrogen (E) and progestin (P), whereas leiomyoma main population readily grew under these conditions. E+P induced minimal growth of leiomyoma side population co-cultured with myometrial

cells maintained in a separate insert. Leiomyoma side population showed a robust growth when in direct contact with myometrial cells in culture; E+P further stimulated this. Xenografts made of leiomyoma side population and myometrial smooth muscle cells grew to relatively large tumors (2.02±0.46 mm^3), whereas leiomyoma main population/myometrial smooth muscle cell xenografts produced smaller tumors (0.29±0.10 mm^3, $p<0.01$, n=8 mice/patients). mRNA levels for estrogen receptor-a, progesterone receptor and smooth muscle cell markers (aSMA, calponin, SM22a) were strikingly lower ($p<0.05$) in leiomyoma side population compared with main population cells of leiomyoma tissues. p16 and p27 protein levels indicated cell cycle dormancy in side population and increased senescence in main population. Our data suggest that the leiomyoma side population with stem/reservoir cell characteristics is necessary for ovarian steroid-dependent in vivo growth of leiomyoma tumors. Strikingly lower estrogen or progesterone receptor levels in side population cells suggests an indirect paracrine effect of steroid hormones on stem cells via neighboring mature cells. NICHD 5P01HD057877, 1R01HD064402.

O-097

Intrauterine Hypoxia Generates Oxidative Stress and Impairs Cytochrome Oxidase (CCO) Activity in Fetal Guinea Pig Organs. Yazan M Al-Hasan,[1] LaShauna C Evans,[2] Gerard A Pinkas,[1] Erinne R Dabkowski,[3] William C Stanley,[3] Loren P Thompson.[1] *[1]Depts. of Obstet, Gynecol, & Repro Sci, Univ. of Maryland; [2]Physiology, Univ. of Maryland; [3]Medicine, Univ of Maryland, Baltimore, MD.*

Chronic intrauterine hypoxia generates oxidative stress in fetal organs, contributing to growth restriction and organ dysfunction. Oxidative stress generates lipid peroxidation and produces malondialdehyde (MDA), a potent inhibitor of CCO, a complex III enzyme of the mitochondrial electron transport chain. We hypothesized that chronic hypoxia inhibits CCO activity via oxidative stress in fetal organs. **Methods.** Pregnant guinea pig sows were exposed to either normoxia (NMX) or hypoxia (10.5% O_2, 14d, HPX) in the presence/absence of the antioxidant, N-acetylcysteine (NAC), via their drinking water. At near term, anesthetized fetuses were delivered via hysterotomy, and fetal livers (N=8-10 each grp), hearts (N=5), lungs (N=9-12), and forebrains (N=12) were harvested, stored frozen, and isolated mitochondrial fractions obtained. We measured the effect of HPX on CCO activity (oxidation of cytochrome c) and two factors known to regulate CCO activity, MDA levels (TBARS assay; nmol MDA/mg prot) and CCO subunit 4 (COX4, Western blot). **Results.** HPX increased ($P<0.05$) MDA levels in fetal livers [1.27±0.13 vs 1.8±0.07 (NMX vs HPX, respectively)], hearts [1.01±0.05 vs 1.73±0.17], and lungs [0.66±0.04 vs 0.81±0.03] but had no effect in fetal brains (0.64±0.08 vs 0.70±0.14). HPX reduced ($P<0.05$) CCO activity in fetal livers (0.15±0.01 vs 0.11±0.01), hearts (0.78±0.03 vs 0.55±0.07) and lungs (0.27±0.01 vs 0.22±.02) but had no effect in fetal brains (0.21±0.01 vs 0.21±0.01). HPX reduced ($P<0.05$) COX4 expression in fetal livers (15%), lungs (37%) and brains (26%) but not in fetal hearts despite the reduced CCO activity. NAC prevented ($P<0.05$) both the HPX-induced increase in MDA levels and the decrease in CCO activity in fetal livers, hearts, and lungs. Neither MDA nor CCO activity of HPX brains were affected by NAC. NAC restored ($P<0.05$) the COX4 expression in HPX fetal livers and lungs to NMX controls but had no effect in fetal brains. **Conclusions.** Fetal HPX regulates CCO activity by increasing MDA levels via oxidative stress. The role of COX4 in regulating CCO activity varies among different organs. Thus, fetal HPX may induce organ dysfunction by inhibiting mitochondrial enzyme activity that is reversible with antioxidant treatment. (NIH HL49999/LT; NIH HL72757/YA).

O-098

C-Type Natriuretic Peptide Is a Potent Endogenous Mediator of Ductus Relaxation That Stimulates a Feed-Forward Mechanism To Perpetuate PDA. Quentin Reuter, Noah Ehinger, Naoko Brown, Stan Poole, James C Slaughter, Tianbing Ding, Bibhash Paria, Robert Cotton, Jeff Reese. *Pediatrics, Vanderbilt University, USA.*

Background

Natriuretic peptides (NP) are released from the heart and endothelium in response to volume overload. They act through guanylyl cyclase receptors Npr1 (ANP, BNP) and Npr2 (CNP), resulting in diuresis and vasodilation. Infants with persistently patent DA (PDA) have increased levels of ANP and BNP. The L-to-R shunt of PDA overloads the heart causing release of NPs, but it is unknown whether NPs play a pathophysiologic role in maintaining a PDA.

Objective

We hypothesized that NPs, acting on Npr1 and 2, have direct vasodilatory effects on DA tone, both *in vitro* and *in vivo*.

Methods

QPCR specific for Npr1, 2, and 3 was performed on DA segments of d15 to P1 mice. Pressure myography was used to determine the effect of NPs on DA tone *in vitro*. We compared preterm vs term and WT vs Npr KO DAs treated with NPs, in both fetal and newborn O2 conditions. Physiologically closed P1 DAs were treated with NPs. *In vivo* studies included ELISA of serum NP levels in mouse models of PDA (PGE-treated newborns and Cox1/Cox2 dKO offspring) vs. control newborns; and imaging of DAs in newborns treated with injections of PGE2, ANP, BNP, or CNP, vs. control. Data were analyzed by t-test, ANOVA, or linear mixed models regression.

Results

QPCR showed stage specific expression of Npr1, 2, and 3 in the DA. Arteriography studies showed that all three NPs relax the DA with CNP>>ANP, BNP ($p<0.01$) in d15 vs d19 (term) DAs ($p<0.01$), in fetal or newborn O2. Npr1 KO DAs had decreased response to ANP and BNP ($p<0.01$), but strong vasodilation to CNP. Npr2 KO DAs had decreased response to CNP, but relaxed with ANP. Pretreatment with the Npr1 inhibitor A71915 partially blocked the response to ANP and BNP. CNP>ANP reopened the physiologically closed P1 DA *in vitro*. Mouse models of PDA had increased serum CNP compared to controls. Postnatal ANP and CNP injections maintained DA patency at 4 hrs, similar to PGE2.

Conclusions

All three NPRs are expressed in the fetal and newborn DA. CNP>>ANP, BNP, acting via specific NPRs, dilated the mouse DA *in vitro*, and maintained its patency *in vivo*. In the presence of congestive failure, CNP may be a potent mediator of PDA. These findings suggest both that NPs could be used to maintain a PDA in infants with cyanotic heart lesions, and NPR antagonists could help close a PDA that is refractory to treatment.

O-099

Multidrug Resistance in the Developing Blood-Brain-Barrier (BBB): Interactions between Cytokines and Glucocorticoids (GCs). Majid Iqbal,[1] Hay Lam Ho,[1] Melanie C Audette,[1] Sophie Petropoulos,[1] William Gibb,[2] Stephen G Matthews.[1] *[1]Physiology, Obstetrics & Gynecology and Medicine, University of Toronto, Toronto, ON, Canada; [2]Obstetrics & Gynecology and Cellular & Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.*

Objective: P-glycoprotein (P-gp) protects the developing fetal brain from a wide range of xenobiotics present in maternal circulation. Cytokines (released during infection) inhibit P-gp, but it is unknown how cytokines can affect fetal brain susceptibility in pregnancies complicated by infection. Infection accounts for ~40% of preterm labor (PTL) risk, for which pregnant women receive synthetic GCs (sGCs). P-gp is stimulated by sGCs, but it is unknown how prenatal sGC exposure (with cytokines) alters fetal BBB multidrug resistance. We hypothesized that: 1) cytokines inhibit P-gp function in brain endothelial cells (BECs); 2) sGC exposure alters subsequent BEC P-gp response to cytokines. Methods: Brain microvessels (BMVs) and BECs were isolated from gestational day (GD) 50, 65 and postnatal day (PND) 14 male guinea pigs. BMVs and BECs were also isolated from GD50 fetuses exposed in utero to dexamethasone (sGC; 1 mg/kg), or vehicle (VEH) on GD48/49. Confluent BEC cultures were treated with 1-10,000 pg/mL of interleukin-1β (IL-1β), interleukin-6 (IL-6), or tumor necrosis factor α (TNF-α) for 1-24h, and P-gp function was assessed (calcein-AM; 1 μM). P-gp and IL-6 receptor (IL-6R) protein were measured in BMVs. Results: IL-1β, IL-6 and TNF-α all reduced P-gp function in BECs derived from PND14 ($P<0.01$). These inhibitory effects were not seen in cells derived from GD50 fetuses, but were present in GD65 BECs ($P<0.01$). In contrast to naive GD50 BECs, GD50 DEX-exposed fetal BECs displayed enhanced responsiveness to the inhibitory effects of cytokines ($P<0.01$). Prenatal DEX exposure increased P-gp and IL-6R protein in BMVs, by 2 to 3-fold, respectively.

Conclusions: Our data suggests that a developmental window exists for cytokine regulation of P-gp function. This window is widened by prenatal exposure to sGCs. Near-term, the P-gp-mediated fetal BBB protection is reduced during infection, potentially leaving the fetal brain susceptible to teratogens. Prenatal sGC exposure appears to further promote this susceptibility. Greater consideration may be needed when administering sGCs for risk of PTL due to infection; particularly to women on medications that are known P-gp substrates. Funded by: Canadian Institutes for Health Research.

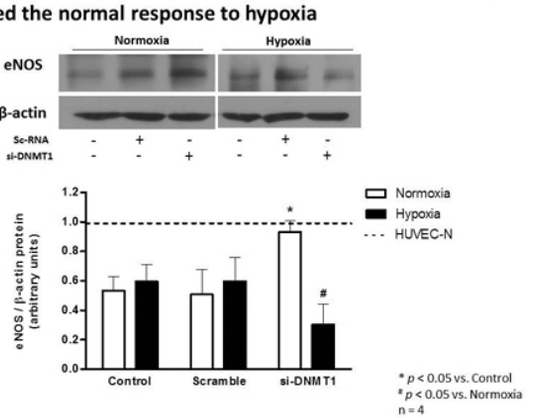
Friday

O-100

The Hypoxic Phenotype of IUGR Placental Endothelial Cells Is Reverted by DNMT1 Silencing. Evidence for Altered Epigenetic Vascular Programming. Bernardo J Krause,[1] Luis Sobrevia,[1] Mark A Hanson,[2] Paola Casanello.[1] [1]*Division of Obstetrics & Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile;* [2]*Developmental Origins of Health and Disease Division, University of Southampton, Southampton, United Kingdom.*

Human umbilical vein endothelial cells (HUVEC) from Intrauterine Growth Restriction (IUGR) placentae present an absent response to hypoxia, along with decreased expression of endothelial NO synthase (eNOS) and increased expression of Arginase-2 (Arg-2). In normal endothelial cells (EC) eNOS expression is strongly regulated by epigenetic mechanisms, however it is not known if they are involved in the altered expression of eNOS and Arg-2 in IUGR HUVEC. We studied the DNA methylation pattern in the promoter region of eNOS and Arg-2 genes, and the role of heritable DNA methylation machinery in the abnormal response to hypoxia in these cells. Methods. HUVEC were obtained from Normal (N) and IUGR placentae. Protein and mRNA levels were determined by Western blot and RT-PCR. Specific CpG methylation of eNOS (10 CpGs) and Arg-2 (12 CpGs) gene promoters was determined by pyrosequencing of bisulfite treated DNA. DNA-methyl transferase 1 (DNMT1) was silenced and their response to hypoxia (2% oxygen) was tested. Results. Protein and mRNA levels of eNOS were lower, whilst Arg-2 levels were higher in IUGR vs N-EC. In IUGR-HUVEC only CpG -352 within the eNOS promoter showed altered methylation (14.4 ±2.6%) vs N-HUVEC (6.5 ±1.3%), whilst there were no changes in the Arg-2 promoter methylation status. In IUGR-HUVEC, silencing of DNMT1 increased the mRNA and protein levels of eNOS in normoxia and rendered these cells sensitive to hypoxia.

In IUGR-HUVEC, DNMT-1 silencing increased expression of eNOS and restored the normal response to hypoxia



Conclusions. IUGR-EC show altered methylation patterns in eNOS promoter region that could be related with its abnormal expression. Heritable DNA-methylation is implicated in the altered response to hypoxia exhibited by IUGR-HUVEC. Supported by FONDECYT 1080534/1110977, CONICYT ACT-73 (PIA) & AT-24100107, Chile. MHA is supported by BHF. BK holds a CONICYT-PhD fellowship.

O-101

Microarray Analysis of Gene Expression in the Ovine Fetal Cerebral Cortex Ontogeny in Late Gestation Suggests the Development of Myeloid-Derived Cells with Tolerogenic Functions. Maria B Rabaglino, Charles E Wood. *Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*

Remarkable physiological changes occur in the fetal brain approaching the end of gestation. Our lab has employed the microarray technique to measure the global gene expression in the ovine fetal cerebral cortex at 80, 100, 120, 130, 145 days of gestational life (the gestation term is ~147 days) and 1 day of extra-uterine life. The data was analyzed using the Empirical Bayes principle to rank the genes according to how well they fit an increased –or decreased-expression pattern. Gene expression was confirmed by qRT-PCR. Higly ranked genes with increased expression pattern were GFAP (marker for astrocytes) MBP (important for myelination), CD14, CD86 or B7.2 and CD11b (microglial markers). Microglia cells are not terminally differentiated cells along the myeloid lineage (called prodendritic cells) and can be skewed to a dendritic cell (DC) phenotype if they are exposed to CSF-1. CSF-1, IL34 (CSF-1R ligand) and CSF1R were increased. DC markers like CD1d, CD83 and CD32 or FCGRIIB were increased. The pattern of gene expression suggested increasing tolerogenic functions in late gestation. FCGRIIB, for example, is an inhibitory receptor on DC that can regulate T-cell tolerance and promotes T-regulatory cell induction by increasing IL10 production. Both IL10 and TGFb, another cytokine involved in T-regulatory cell induction, had an increased expression pattern. In contrast, a cytokine that had a decreased expression pattern was IL6. IL6 is involved in the differentiation of Th17 cells, which have been related with experimental autoimmune encephalitis (EAE). EAE can be induced in the Lewis rat by active immunization with MBP and can be prevented by injection of encephalitogenic MBP peptide-pulsed bone marrow DC before MBP immunization. Critically important for the progress of EAE is expression of CD24, which is required for the optimal local T cell clonal expansion in the CNS. Interestingly, CD24 mRNA expression was remarkably decreased from 80 days of gestation to 1 day of extra-uterine life. Together, our results suggest that a myeloid cell type -probably DC- is differentiated from the local microglia. These cells could be destined to exert tolerogenic functions and avoid an autoimmune reaction of T cells against self-proteins that are being produced at a highly increased rate by the end of gestation, like MBP.

O-102

Activated Renin-Angiotensin System in the Adrenals of Offspring after Exposure to Maternal Dietary Restriction during the Periconceptional Period. Song Zhang,[1] Amreet Gill,[1] Janna Morrison,[1] Leewen Rattanatray,[1,2] Severence MacLaughlin,[1] David Kleemann,[3] Simon Walker,[3] Caroline McMillen.[1] [1]*Sansom Institute for Health Research, University of South Australia, Australia;* [2]*Discipline of Physiology, University of Adelaide, Australia;* [3]*Turretfield Research Centre, SARDI, Australia.*

It has previously been shown that periconceptional dietary restriction in normal and overweight ewes results in increased adrenal weight and cortisol response to stress in postnatal lambs, however the mechanisms are not known. The activation of intraadrenal renin-angiotensin system (RAS) has been demonstrated to promote adrenal growth and steroidogenesis through angiotensin II type I receptor (AT1R) activation. Angiotensin-converting enzyme 2 (ACE2) is an ACE homologue that cleaves angiotensin I and II to smaller peptides. We have therefore hypothesized that periconceptional dietary restriction in normal and overweight ewes activates the adrenal RAS in postnatal lambs.

Donor ewes were assigned to one of 4 nutritional treatment groups pre-conception: CC, 100% metabolisable energy requirement (MER) for 5 months; CR, 100%MER for 4 months followed by 70% MER for 1 month; HH, *ad libitum* (180% MER) for 5 months; HR, *ad libitum* for 4 months followed by 70%MER for 1 month. At 6-7 d post-conception, single embryos were transferred into normal weight recipient ewes. Post mortem was conducted and tissues collected from lambs at 4 months of age.

Adrenal cortical area and specifically the zona fasciculata-reticularis (ZF-ZR) area were each higher ($P<0.05$) in CR than CC and HH. In females, there was a significant decrease ($P<0.05$) in adrenal ACE2 mRNA expression in CR and HH than CC by qRT-PCR. Immunostaining for ACE, ACE2 and AT2R was localized throughout the zona glomerulosa (ZG) and ZF-ZR of the adrenal cortex. AT1R immunostaining was primarily localized in the adrenal cortex with higher staining present in the ZF-ZR than the ZG. ACE and AT1R staining intensity in both the ZG and ZF-ZR was higher ($P<0.05$) in CR and HR than CC. These results demonstrate that there is an activation of the intraadrenal RAS, through an increase in ACE and AT1R and a decrease in ACE2, in the adrenal cortex of offspring exposed to dietary restriction during the periconceptional period. Thus dietary restriction in either normal weight or obese ewes results in long term changes in the intraadrenal RAS which may contribute to increased adrenal growth and stress responsiveness in postnatal life.

O-103

Cardiac Hypertrophy and Fibrosis in Early Adulthood Following Intrauterine Growth Restriction. Jennifer A Thompson,[1,2] Robert Gros,[1,3] Timothy RH Regnault.[1,2] [1]*Physiology and Pharmacology, The University of Western Ontario, London, ON, Canada;* [2]*Children's Health Research Institute, LHRI, London, ON, Canada;* [3]*Program in Vascular Biology, Robarts Research Institute, London, ON, Canada.*

Introduction Placental insufficiency (PI) is a common complication of human pregnancy, leading to intrauterine growth restriction. We have previously shown that PI leads to altered aortic development in the growth impaired fetus and stiffening at 15 months of age. Aortic stiffening causes disturbance in hemodynamics with an augmentation in systolic load. The heart compensates through hypertrophic growth which involves remodeling of the extracellular matrix. Eventually, excess collagen accumulation leads to fibrosis and cardiac

dysfunction. The goal of the present study was to examine markers of aortic stiffness and cardiac compensation in growth restricted guinea pig offspring in early adulthood. **Methods** PI was induced in pregnant guinea pigs by uterine artery ablation at mid-gestation. Pups were allocated to a normal birth weight (NBW) and low birth weight (LBW) group. Pups were sacrificed at 4 mo of age. Cross-sections of thoracic aortae were stained with Hematoxylin-eosin and a 2% Orcein solution for identification of elastic fibres. Cardiac cross-sections were stained with 1% Sirius red with picric-acid to analyze the extent of collagen deposition. Statistical analyses were performed using an un-paired t-test. **Results** The birth weights of LBW guinea pigs were 20% lower than those of NBW guinea pigs. At 4 mo of age, the volume fraction of elastic lamellae in the aorta was lower in LBW compared to NBW offspring ($p < .05$). Hematoxylin-eosin staining showed that impaired fetal growth was not associated with lesion formation at 4 mo of age. The relative heart weights in LBW offspring were greater than those of NBW offspring ($p < .05$). The % area stained for fibrillar collagen in the left ventricle ($p < .05$) was increased in LBW vs. NBW offspring. **Conclusions** The reduction in volume of elastic lamellae in 4 mo-old LBW offspring suggests that the aortic stiffening previously found in 15-mo offspring, has manifested by this earlier age. Cardiac compensation is already apparent at this age, as reflected by increased heart weight and collagen accumulation. Thus, structural abnormalities established *in utero* leading to hemodynamic imbalance and adverse cardiac responses are apparent in early adulthood and may lead to heart disease in growth restricted offspring.

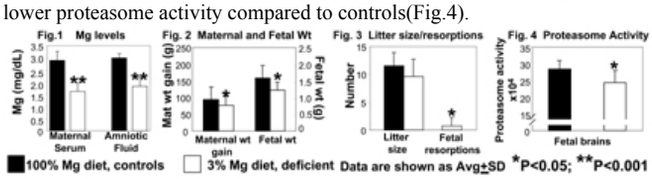
O-104

Magnesium (Mg) Deficiency In Utero Restricts Fetal Growth and Impairs Brain Proteasome Function. Madhu Gupta,[1] Neeraj Desai,[2] Amanda Roman,[2] Burton Rochelson,[2] Xiangying Xue,[1] Christine Metz.[1] *[1]Maternal-Fetal Medicine, Feinstein Institute of Medical Research, Manhasset, NY, USA; [2]Maternal-Fetal Medicine, North Shore-LIJ Health System, Manhasset, NY, USA.*

Objective: We examined the effect of Mg deficiency during pregnancy on maternal and fetal outcomes, including brain dysfunction because 1) maternal $MgSO_4$ treatment is neuroprotective in the fetus, 2) Mg supplementation improves cognition in rats, and 3) pregnancy increases Mg demands.

Methods: Female Wistar rats maintained on either normal (control, 100% Mg) or Mg-deficient (3% Mg) diets from gestational day 1(GD1) throughout pregnancy were euthanized on GD19 (n=10/group). Maternal-fetal outcomes, as well as serum and amniotic fluid Mg levels were assessed. Fetal brains were analyzed for gene expression using Illumina gene chips+QPCR and ATP-Mg^{2+}dependent proteasome activity. Data were analyzed using Students t tests (*P<0.05; **P<0.001).

Results: Mg deficiency in utero significantly reduced maternal serum and amniotic fluid Mg(Fig.1). Average maternal weight gain and fetal pup weight were significantly lower among the Mg-deficient rats(Fig.2). While litter sizes were similar in the 2 groups, only Mg-deficient dams had fetal resorptions(Fig.3). Numerous genes associated with brain development, including *Hoxb7, Hoxb8, Picalm, Pnck, Snca, Eno2,* and *Dnmt3a* were differentially expressed in Mg-deficient fetal brains vs. controls. Mg deficient fetal brains had significantly lower proteasome activity compared to controls(Fig.4).



Conclusion: 1) Mg deficiency restricts fetal growth, a risk factor for neurological dysfunction and cerebral palsy, a condition for which maternal $MgSO_4$ administration is protective. 2) Numerous genes that regulate brain development and synaptic plasticity were differentially expressed in Mg deficient fetal brains. 3) Mg deficiency also reduced brain ATP-Mg^{2+}dependent-proteasome activity, which is critical for removing mutant/damaged proteins implicated in neurological dysfunction, as well as nerve and muscle disorders. We are examining the effects of Mg deficiency in utero on cognitive abilities and behavior in the offspring.

O-105

PR-A inhibits PR-B-Mediated Anti-Inflammatory Actions of Progesterone in Human Myometrial Cells and Induces a Pro-Inflammatory/Pro-Labor State. Huiqing Tan, Lijuan Yi, Sam Mesiano. *Reproductive Biology, Case Western Reserve University, Cleveland, OH, USA.*

Progesterone (P4) promotes uterine relaxation for most of pregnancy via its interaction with the nuclear P4 receptors (nPRs), PR-A and PR-B, in myometrial cells, and labor is induced by inhibition of nPR-mediated P4 actions (i.e., functional P4 withdrawal). We proposed that P4 promotes myometrial relaxation via its interaction with PR-B and that this activity is inhibited at parturition by increased expression of PR-A, which represses PR-B-mediated transcriptional activity. Studies have also shown that labor is an inflammatory process. It is hypothesized that P4 blocks labor by acting as an anti-inflammatory agent, in part by increasing expression of inhibitor of κBα (IκBα) that sequesters and inhibits the activity of the nuclear factor-κB (NFκB) transcription factor complex. In this study we examined whether the PR-A:PR-B ratio in human myometrial cells affects the capacity for P4 to modulate basal and cytokine-induced expression of IκBα and the NFκB dependent pro-inflammatory genes PG-endoperoxide synthase 2 (PTGS2) and interleukin-8 (IL-8) in hTERT-HMA/B cells, an immortalized human myometrial cell line in which the levels of PR-A and PR-B can be experimentally controlled. hTERT-HMA/B cells expressing various PR-A:PR-B levels were exposed to P4 (100 nM) or vehicle for 24h and then challenged with E-coli lipopolysaccharide (LPS) (10 μg/mL) for 6h. Quantitative RT-PCR was then used to assess effects on expression of IκBα, PTGS2 and IL-8. In cells expressing mainly PR-B (i.e., PR-A:PR-B < 1) P4 increased expression of IκBα and repressed the capacity for LPS to induce expression of PTGS2 and IL-8 ($P < 0.05$). In cells expressing mainly PR-A (i.e., PR-A:PR-B > 1), P4 increased basal and LPS-induced expression of PTGS2 and IL-8 and inhibited PR-B induced expression of IκBα ($P < 0.05$). Our data suggest that during most of human pregnancy (when myometrial cells are PR-B-dominant) P4 promotes myometrial cell relaxation through PR-B-mediated anti-inflammatory actions via increased IκBα expression. At parturition increased expression of PR-A in myometrial cells inhibits PR-B-mediated anti-inflammatory actions. We propose that a PR-A:PR-B threshold exists whereby actions of P4 change from PR-B-mediated anti-inflammatory/pro-relaxation to PR-A-mediated pro-inflammatory/pro-labor and that this initiates a positive feedback pro-inflammatory state within the myometrium that induces labor.

O-106

Revealing Mechanisms for Adverse Neurobehavioral Outcomes from Exposure to Prenatal Inflammation. Monique E Maubert, Amy G Brown, Michal A Elovitz. *Maternal and Child Health Research Program, Department of Obstetrics and Gynecology, CRRWH, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.*

Introduction: Exposure to prenatal inflammation in the preterm or term period is associated with a spectrum of neurobehavioral disorders ranging from cognitive delay to cerebral palsy to autism. We hypothesize that exposure to intrauterine inflammation results in specific disruptions in gene pathways controlling neuronal and glial development and that these abnormalities persist into the postnatal period.

Methods: We created a custom array to assess a molecular profile of neuronal and glial injury (MPNGI) with 30 genes representing specific pathways. Using a mouse model of intrauterine inflammation, low dose LPS (50 ug/dam) or saline (NS) was infused into the uterine cavity on E15. Fetal brains (FB) were collected 48 hours post-infusion. FBs were used for RNA or to create neuronal cultures (NC). In separate studies, dams were allowed to deliver at term. Pups on postnatal day 10 (P10) were euthanized and the prefrontal cortex (PFC) and cerebellum (CBL) were harvested. RNA from NC, FB at 48 hours, PFC and CBL on P10 from LPS and NS dams were used to assess the MPNGI using qPCR.

Results: In NC, genes involved in neurite outgrowth and neuronal differentiation were differentially expressed (ATF3, BASP1, CDK5r1). In FB, 48 hrs after exposure, similar genes/pathways were involved as well as neuronal development (BASP1, CDK5rap1, CNTN3, NRCAM, RELN). On P10, there were changes in gene expression noted in the CBL but there were dramatic changes noted in the PFC (Table 1).

Friday

Table 1: Fold change in mRNA in P10 brain regions exposed to low dose prenatal inflammation.

Gene	Pathway	PFC		CBL	
		Fold Change	P value	Fold Change	P value
MCT1	Glial differentiation	-5	0.01		NS
PLP1	Glial differentiation	-2.1	0.02		NS
GFAP	Glial differentiation	-3.3	0.01		NS
MBP	Glial differentiation		NS	-34	0.04
MAG	Glial differentiation	-4.7	0.03		NS
S100B	Glial differentiation	-2	0.008		NS
POU4F1	Synaptogenesis	-9	0.04		NS
APOE	Regulation of synaptic plasticity	-3.3	0.007		NS
YWHAH	Regulation of synaptic plasticity	-3.9	0.01		NS
TUBB3	Neuronal migration	-9	0.03		NS
NRCAM	Neuronal Development	-5.5	0.03		NS
NCAM1	Neuronal Development	-4.5	0.04		NS
RELN	Neuronal Development	-4.1	0.046		NS
CDK5r1	Regulation of neuronal differentiation	-5.5	0.04		NS
CDK5rap3	Regulation of neuronal differentiation	-3.4	0.003		NS
CDKrap1	Regulation of neuronal differentiation	-3	0.004		NS
CDKrap2	Regulation of neuronal differentiation	-4.3	0.04		NS
CNTN3	Neurite outgrowth	-2.8	0.04		NS
ATF3	Neurite outgrowth	-4	0.01		NS
BASP1/CAP23	Neurite outgrowth	-6.5	0.03	-8.2	0.04
GRIN1	Glutamate transport		NS	-7.5	0.05
EAAT1	Glutamate transport	-3.4	0.006		NS
NGB	Neuroprotective	-3	0.02		NS
TIMP3	Neuroprotective	-5.8	0.01		NS

PFC=prefrontal cortex; CBL=cerebellum;
Fold Change is mean expression in LPS-exposed by expression unexposed brains

Conclusions: Exposure to low dose intrauterine inflammation results in specific injury to the fetal brain. This injury persists in the postnatal brain, most notably in the PFC. The diverse effect of prenatal inflammation on many gene pathways that dictate neuronal and glial development likely contributes to the spectrum of neurobehavioral disorders observed in exposed offspring. These studies further demonstrate a causal relationship between prenatal inflammation and direct neuronal and glial injury.

O-107

A New Role for Monocytes in Modulating Myometrial Inflammation during Labour. K Srikhajon,[1] D Preechapornprasert,[2] O Shynlova,[3] B Chanrachakul,[1,2] SJ Lye.[3] *[1]Mol. Med., Mahidol Univ., Bangkok, Thailand; [2]OB-GYN, Ramathibodi&Bumrungrad Hospital, Bangkok, Thailand; [3]Samuel Lunenfeld Res. Inst., Mount Sinai Hospital, Univ.Toronto, Canada.*

Background: We previously showed that the laboring myometrium is a major source of CCL2, a potent monocyte chemoattractant. The increase in CCL2 expression coincides with an influx of monocytes into myometrium which we propose contributes to the initiation of labour through induction of physiologic inflammation. We hypothesized that monocytes and myometrial myocytes(USMCs) act synergistically to induce the cytokines that produce this inflammatory response.

Methods: Primary human (h)USMCs cells were prepared from term, non-laboring myometrial biopsies and cultured at 180,000 cells/well. THP-1 human monocytic cells and hUSMCs were cultured separately in media with 10% FBS but serum-starved for 24 hours prior to study . Co-cultures was constructed by seeding THP-1 cells at 50,000-400,000 per well, either directly in contact with confluent hUSMCs, or separated from the hUSMCs in a culture insert. Supernatants were collected every 24 hours for 3 days and analyzed by BioPlex. On day 3 hUSMCs were harvested , RNA extracted and the expression of cytokines quantified by RT-PCR. 1-way ANOVA and Dunnett's multiple comparison tests were used to compare cytokine gene expression by hUSMCs cultured in direct or indirect contact with THP-1 monocytes.

Results: Surprisingly, rather than increase cytokine expression, the presence of monocytes significantly decreased CCL2 synthesis by hUSMCs. The decrease more pronounced with increasing number of monocytes and when monocytes were in direct contact with hUSMCs. CCL2 inhibition was apparent at 24 hours, but greater after 3 days in co-culture.

Conclusion: Our data suggest a novel model in which monocytes are first recruited to the myometrium by CCL2 to contribute to physiologic inflammation of labour. After completing transmigration and coming into contact with hUSMCs the monocytes inhibit further CCL2 expression, thus providing a means to prevent the adverse effects of an uncontrolled inflammatory response.

CCL2 levels (normalized to controls) in culture supernatants

Conditions	24 h	48 h	72 h
hUSMC control culture	100≠	100≠	100≠
hUSMC + THP-1(400,000)	47.5 ± 5.02+	15.60 ± 5.74+	5.81 ± 5.88+
hUSMC + THP-1 (200,000)	76.27 ± 1.27	50.14 ± 11.69*	36.23 ± 12.99*
hUSMC + THP-1 + insert	76.76 ± 7.15	50.18 ± 14.48*	31.58 ± 9.34*

Different symbols denote sig. diff. p< .05.

O-108

15dPGJ2 Reduces NF-κB in Peripheral Blood Mononuclear Cells and Reduces the Production of the Pro Inflammatory Cytokines IFNγ and TNFα in T Helper Cells. Lynne Sykes,[1] Sathana Ponnampalan,[1] Xiao J Yap,[1] TG Teoh,[2] Phillip R Bennett.[1] *[1]IRDB, Imperial College London, United Kingdom; [2]Obstetrics and Gynaecology, Imperial College NHS Trust, United Kingdom.*

NF-κB is a pro inflammatory transcription factor which also controls the expression of labour associated genes. NF-κB is activated in the myometrium at term and in labour. 15dPGJ2 inhibits NF-κB in human myocytes, delays preterm labour and improves pup mortality in LPS treated mice. Preterm labour is often associated with a pro inflammatory profile, and administration of 15dPGJ2 could have anti inflammatory effects on human peripheral blood mononuclear cells (PBMCs). We examined NF-κB activity in PBMCs at different time points in pregnancy and in labour, and the effect of 15dPGJ2 on it. We also examined the production of PMA stimulated IFNγ and TNFα in the presence or absence of 15dPGJ2.

Blood was taken from non pregnant controls, women at 28 weeks gestation, and at term (pre labour and in labour). Phospho-p65 was detected using western analysis, and intracellular PMA/Ionomycin stimulated IFNγ and TNFα of CD4 positive cells (T helper cells) were detected by flow cytometry.

No difference was seen in phospho-p65 between non pregnant and pregnant or term pre labour and in labour PBMCs. However there was a reduction in the proportion of Th cells producing PMA stimulated IFNγ and TNFα in pregnancy compared with non pregnant controls. 10% of Th cells produce IFNγ in non pregnant women, reducing to 6.5% at 28 weeks, 5% at term and 5.5% at term in labour (p=0.05, 0.01, 0.02 respectively). 20% of Th cells produce TNFα in non pregnant women, reducing to 14% at 28 weeks, 15% at term and 13% at term in labour. 15dPGJ2 reduced basal (p=0.02) and PMA stimulated phospho p65 (p=0.03), and led to a reduction in PMA stimulated IFNγ and TNFα at all gestations. 15dPGJ2 reduced the mean fluorescence intensity (MFI) of IFNγ from 101 to 53 in non pregnant women (p=0.01), 115.7 to 52.5 at 28 weeks (p=0.04), and 110.1 to 60.7 at term (p=0.02). A reduction in MFI of TNFα was also seen, but did not reach statistical significance.

15dPGJ2 suppresses NF-κB and the expression of pro inflammatory cytokines in T helper cells *in vitro*. This reveals a potential beneficial systemic effect to complement the previously identified local effect of NF-κB inhibition in myocytes. We propose that 15dPGJ2 should be considered as a potential therapeutic agent for the prevention of preterm labour and reduction of neonatal morbidity.

O-109

Broad Spectrum Chemokine Inhibitor Delays Infection-Induced Preterm Delivery in Mice. Oksana Shynlova,[1] Anna Dorogin,[1] Yunqing Li,[1] Tam Lye,[1] Stephen Lye.[1,2,3] *[1]Samuel Lunenfeld Res Institute, Mt Sinai Hospital, Toronto, Canada; [2]Dep of Physiology, University of Toronto; [3]Dep of Ob/Gyn, University of Toronto.*

Introduction: Systemic and intrauterine infection increases cytokine biosynthesis in both fetal and maternal tissues and results in preterm delivery. We investigated the ability of a Broad Spectrum Chemokine Inhibitor (BSCI) to prevent infection-induced preterm delivery (PTD) in mice.

Methods: Pregnant CD-1 mice (n=144) were used on day 15.5 gestation. Study A – Systemic Infection Model: mice received 50μg/mouse intraperitoneal injection of lipopolysaccharide (LPS-IP) or sterile saline (Control). Study B – Intrauterine (IU) Infection Model: mice were subjected to a mini-laparotomy and 50μg/mouse LPS was injected into right uterine horn (LPS-IU); control animals received 100μl IU injection of sterile saline. In both studies, half the mice also received BSCI (BN83470, 10mg/kg sc - Funxional Therapeutics Ltd, UK) 24h prior to and immediately before LPS administration. For both studies A and B two sets of animals were used to assess, (1) the impact of LPS alone or LPS+BSCI on injection-to-delivery interval, maternal morbidity, fetal survival rate, fetal and placental weights; and (2) amniotic fluid and maternal plasma (MP) cytokine levels (BioPlex) and placenta / maternal liver tissue cytokine mRNA levels (by RT-PCR) and to determine the effect of BSCI on labour-induced genes and production of inflammatory cytokines IL6, TNFα, IL1β, GM-CSF in response to LPS.

Results: 1) LPS administration induced PTD in 100% of LPS-IP group and 40% of LPS-IU group. Co-administration of BSCI and LPS significantly delayed ($p<0.05$) PTD in both study group (64% vs 100% in LPS-IP group and 25% vs 40% in LPS-IU group). 2) Fetuses in which PTD was delayed till term were live and had normal placental and fetal weight. 3) IL6, TNFα, IL-1β, GM-CSF were markedly elevated in MP and liver 2h after LPS administration (IP or IU). BSCI significantly attenuated pro-inflammatory cytokine synthesis but only in

LPS-IP group. LPS (both IP and IU) induced placental inflammation (increased IL6, IL1α, IL1β and MCP1 mRNA levels), which was not attenuated by BSCI. **Conclusions:** BSCI reduces the incidence of LPS-mediated preterm delivery in mice and is associated with reduced cytokine expression in maternal (but not fetal) tissues. These data provide support for efforts to target inflammatory responses as a means of preventing preterm delivery.
Funding: MOD (grant # 21-FY10-204)

O-110

Vitamin D Reduces Monocyte-Induced Expression of Estrogen Receptor α and Oxytocin Receptor Via Regulation of NFκB Pathway in Human Uterine Smooth Muscle Cells. Chandrasekhar Thota,[1] Ramkumar Menon,[2] Ayman Al-Hendy.[1] *[1]Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
BACKGROUND: Infection is one of the main etiologies of preterm birth. African Americans (AA) have been reported to have higher basal inflammation, lower levels of serum vitamin D, a ligand with anti inflammatory properties, and higher incidence of preterm birth. Therefore, infection, during pregnancy in AA, can further increase inflammation and the incidence of preterm birth through an increase in estrogen milieu and contractile profile. However, studies on the role of vitamin D in the regulation of contractile associated factors and NFκB pathway in the myometrium are lacking.
OBJECTIVE: To assess if heightened inflammation affect the expression of estrogen receptor α (ERα), its co activators SRC2 and SRC3, oxytocin receptor, IκBα and NFκBp50 and p65 in uterine smooth muscle (UtSM) cells and to assess the role of vitamin D in their regulation.
STUDY DESIGN: UtSM cells were cocultured with monocyte lineage (THP1 cells; 200k) cells to simulate the condition of inflammation. UtSM cells cocultured with THP1 (200k) cells were treated in triplicates with vitamin D (5, 50 and 150nM) for 24h and 48h. Expressions of ERα, NFκBp50 and p65 in nuclear proteins, oxytocin receptor in whole cell lysate and IκBα in cytosol were assessed using western blot analysis. Expressions of estrogen receptor co-activators SRC2 and SRC3 were measured by real time PCR.
RESULTS: UtSM cells cocultured with THP1 cells showed a significant increase ($p<0.05$) in the expression of ERα, oxytocin receptor, NFκBp50 and p65. Vitamin D treatment caused a significant decrease ($p<0.05$) in THP1 induced ERα, oxytocin receptor, NFκBp50 and p65 expression in UtSM cells at 48h. Coculture did not affect IκBα and SRC3 expression in UtSM cells, but vitamin D treatment significantly decreased ($p<0.05$) their expression. There are no significant changes in SRC2 expression.
CONCLUSION: These findings suggest that monocytes induce while vitamin D treatment reverses the expression of estrogen receptor α, oxytocin receptor NFκBp50 and p65. These results suggest that vitamin D reduces inflammation induced contractile profile through NFκB pathway in uterine smooth muscle cells.

O-111

Increased Mammalian Target of Rapamycin Complex-1 (mTORC1) Signaling Is a Major Contributor to Preterm Birth. Jeeyeon M Cha,[1] Yasushi Hirota,[2] Takiko Daikoku,[1] Sudhansu K Dey.[1] *[1]Division of Reproductive Sciences, Cincinnati Children's Research Foundation, Cincinnati, OH, USA; [2]Department of Obstetrics and Gynecology, University of Tokyo, Tokyo, Japan.*
Many signaling pathways that contribute to tumorigenesis are also functional in pregnancy, although they are dysregulated in the former and tightly regulated in the latter. Transformation-related protein 53 (*Trp53*), which encodes p53, is a tumor suppressor gene whose mutation is strongly associated with cancer. However, its role in female reproduction is poorly understood. We generated mice that harbor a conditional deletion of uterine *Trp53* in female mice and examined their pregnancy outcome. These mice had normal ovulation, fertilization, and implantation; however, postimplantation uterine decidual cells showed terminal differentiation and senescence-associated growth restriction with increased levels of phosphorylated Akt and p21, factors that are known to participate in these processes in other systems. Strikingly, uterine deletion of *Trp53* increased the incidence of preterm delivery with dystocia and fetal death. This condition was corrected by an oral administration of a selective COX2 inhibitor (celecoxib), suggesting that the deletion of uterine *Trp53* induces preterm birth through a COX2/PGF synthase/$PGF_{2\alpha}$ pathway. Upon further analysis, we found that decidual senescence early in pregnancy in conditionally deleted *Trp53* mice is accompanied by heightened mTORC1 (mammalian target of rapamycin complex 1) signaling. Our results show that mTORC1 signaling is a significant contributor of preterm birth with fetal death when seeded early in pregnancy, since this phenotype is rescued by an oral gavage of low dose of rapamycin, an mTORC1 inhibitor. This role of mTORC1 signaling in determining the timing of birth in mice may help us to better understand the mechanism of the timing of birth in humans and develop new strategies to combat preterm birth which is a global problem. In conclusion, our observations underscore what we believe to be a new critical role of uterine mTORC1 signaling in parturition. (This work was funded in parts by the Bill & Melinda Gates Foundation through the Grand Challenges Explorations Initiative, NIH grants (HD12304 and DA06668 to S. K. D.), and a Ruth L. Kirschstein National Research Service Award Fellowship (1F30AG040858-01).

O-112

Role of Myometrial Cell Inflammsaomes in the Control of Human Parturition. Matrika Johnson, Huiqing Tan, Lijuan Yi, Sam Mesiano. *Reproductive Biology, Case Western Reserve University, Cleveland, OH, USA.*
It is generally accepted that parturition is an inflammatory process and that labor is a sequelae of sterile inflammation within the myometrial compartment. In this context the mechanism that initiates and mediates the inflammatory process in the pregnancy myometrium is central to the pathophysiology of human parturition. To address this issue we explored the role of myometrial cell inflammasomes in the process of human parturition. Inflammasomes are multi-protein cytosolic complexes that upon activation by physiologic danger signals (e.g., infection, mechanical or biochemical stress), bind pro-caspase-1 and facilitate its cleavage to active caspase-1. Caspase-1 then converts pro-interleukin (IL)-1β to active IL-1β, which is secreted and acts in an autocrine/paracrine manner to initiate a tissue-level inflammation. Three inflammasomes complexes have been well-characterized: NLRP-1, NLRP-3 and NLRP-4. In this study we determined whether these inflammasomes are expressed by human pregnancy myometrium and whether the onset of labor at term is associated with increased inflammasome activity in myometrial cells. To this end, myometrium was collected from term (37-39 weeks) c-sections performed before (n=4) and after (n=4) the onset of active labor and used to measure: 1) the extent and localization of caspase-1 and NLRP-1, -3 and -4 gene expression; 2) the extent of conversion of pro-cascapse-1 to caspase-1 (i.e., inflammasome activity) and 3) the extent of expression of pro-inflammatory NFκB-responsive genes. We found that term myometrial cells express caspase-1 and the inflammasomes NLRP-1, -3 and -4. Levels of mRNAs encoding NLRP-1 (and its associated protein PYCARD) predominated suggesting that this is the main inflammasome in term myometrium. Expression of the NLRPs was not affected by labor status. Levels of activated caspase 1 significantly ($P < 0.05$) increased in laboring compared with non-laboring myometrium and was associated with increased expression of the pro-inflammatory NFκB responsive genes COX-2 and IL-8. These data, for the first time, show that inflammasomes (mainly NLRP-1/PYCARD) are active in term myometrium and contribute to the inflammatory state that induces labor. Whether inflammasome activation is causal to labor remains uncertain. However, inhibition of myometrial cells inflammasome activity may be a key strategy to suppress preterm labor and prevent preterm labor.

O-113

Abnormal Umbilical Artery Waveforms in the COMT-/- Mouse Model of Preeclampsia Are Normalized by Sildenafil Citrate. Joanna L Stanley,[1,2] Irene J Andersson,[1] Christian F Rueda-Clausen,[1] Rajan Poudel,[1] Colin P Sibley,[2] Sandra T Davidge,[1] Philip N Baker.[1,2] *[1]Obstetrics/Gynecology and Physiology, University of Alberta, Edmonton, AB, Canada; [2]Maternal and Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*
Background: Pregnant mice deficient in the enzyme catechol-O-methyl transferase (COMT-/-) display a preeclampsia-like phenotype and deliver growth-restricted fetuses (1,2). Sildenafil citrate potentiates nitric oxide-mediated vasodilation and is able to normalize pup growth in the COMT-/- model (2). We hypothesized that COMT-/- mice would demonstrate abnormal uterine artery blood flow and function, and that treatment with Sildenafil citrate normalizes pup growth by improving uterine artery blood flow velocity and function.
Methods: Pregnant COMT-/- and control (C57Bl6/J) mice received Sildenafil citrate (0.2 mg/ml in drinking water, over gestational days 12.5-18.5), or normal tap water. Uterine and umbilical artery Doppler waveforms were assessed at day 17.5 with blood pressure (BP) and proteinuria. At day 18.5 uterine artery endothelial function was assessed using wire myography.

Results: COMT-/- mice did not demonstrate increased systolic BP (122 ± 5 vs controls: 125 ± 6 mmHg). Proteinuria was increased in COMT-/- mice (1.7 ± 0.7 vs 0.3 ± 0.1 albumin:creatinine ratio; $p<0.05$). Sildenafil had no effect on BP or proteinuria.

In COMT-/- mice, minimum umbilical artery velocity was markedly reduced versus control (2 ± 6 vs. 20 ± 1 cm/s; $p<0.05$) and reverse umbilical artery blood flow velocity was observed. Both minimum artery velocity (16 ± 2 cm/s; $p<0.05$) and reverse umbilical artery blood flow were normalized following Sildenafil treatment.

No differences in uterine artery waveforms were observed between COMT-/- and control mice. Sildenafil treatment increased in vitro uterine artery sensitivity to methacholine in both control and COMT-/- mice (EC50 =118 ± 53 vs. 16 ± 5 nM control mice and 95 ± 16 vs. 41 ± 8 nM COMT-/- mice; $p<0.05$).

Conclusions: In this model, fetal growth restriction is associated with abnormal umbilical artery waveforms. Sildenafil citrate normalized pup growth (2), normalized umbilical artery Doppler waveforms and enhanced uterine artery vasodilation. These data suggest that Sildenafil citrate can improve fetal growth, predominantly via reducing fetoplacental vascular resistance.

1Kanasaki et al. 2008 Nature 453:1117-21
2Andersson et al. 2011 Repro Sci 18(4):S-227

O-114

Comparison of Cardiovascular Response to Volume Challenge in Nulliparous Women and Previous Preeclamptics. Sarah A Hale,[1] Gary J Badger,[2] Carole McBride,[1] Ira M Bernstein.[1] [1]*Ob/Gyn and Reproductive Sci, University of Vermont, Burlington, VT, USA;* [2]*Medical Biostatistics, University of Vermont, Burlington, VT, USA.*

Background: Our laboratory has hypothesized that preeclampsia results from an intolerance to volume expansion in the face of pregnancy. The current study aimed to evaluate cardiovascular response, outside of pregnancy, to exogenously administered volume in nulliparous women (CTL) compared to prior preterm preeclamptics (prePE). We hypothesized that PrePE would have an increased blood pressure response to the volume challenge. Methods: We enrolled 19 nulliparous women (CTL) and 10 prePE. All evaluations were performed during the follicular phase after an overnight fast. 500mL of lactated Ringers solution was infused through an indwelling antecubital catheter over 10 minutes. Beat-to-beat blood pressure, pulse and cardiac output (CO) were obtained continuously and noninvasively using the Finapres Pro blood pressure monitoring system prior to, during and for 15 minutes after the infusion. Results were averaged over 2 minute time intervals and area under the curve, calculated from the beginning of the infusion until the end of the monitoring period and controlled for baseline value, analyses completed. Data are reported as mean ± SE. $P<0.05$ was considered significant. Results: There were no differences between groups in age, however, BMI, and mean arterial pressure (MAP) tended to be higher at baseline in the previous preeclamptics (BMI: CTL: 24.8 ± 1.3 vs. prePE: 28.9 ± 2.1 kg/m2, $p = 0.09$; MAP: CTL: 87.4 ± 1.5 vs. prePE: 92.6 ± 2.9 mmHg, $p = 0.08$). After controlling for baseline differences there were significant increases in prePE area under the curve for pulse pressure (CTL: 19.0 ± 19.3 vs. prePE: 103.6 ± 31.8 mmHg, $p = 0.02$). MAP response tended to be increased in prePE (CTL: 102 ± 22.2 vs. prePE: 167.9 ± 31.0 mmHg, $p = 0.09$). We did not find any differences in CO, pulse or CO/pulse pressure, an additional measure of arterial compliance. Conclusions: Our data suggest that under conditions of volume loading, a direct challenge to the cardiovascular system, prePEs exhibit blood pressure responses consistent with reduced vascular compliance. These findings underscore the existence of a maladaptive hemodynamic system, apparent even in the absence of pregnancy, that is theorized to be an underlying condition in preeclampsia.

O-115

PKC-Dependent Impairment of Endothelial Ca^{2+} Signaling and EDHF-Mediated Vasodilation in Uteroplacental Arteries from Diabetic Pregnant Rats. Natalia I Gokina, Erika Linder, Karen Oppenheimer. *Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA.*

Objective: Diabetes in pregnancy is associated with fetal abnormalities in part due to reduced uteroplacental blood flow. Increased PKC activity is an essential mechanism of diabetic vascular complications. To characterize the role of PKC in diabetes-induced uterine endothelial dysfunction we: (1) defined the effects of PKC activation or inhibition on EDHF-mediated smooth muscle cell (SMC) $[Ca^{2+}]_i$ and dilator responses to ACh; (2) elucidated PKC dependence of ACh-induced endothelial cell (EC) $[Ca^{2+}]_i$; (3) evaluated the effect of diabetes on PKC activity in uterine arteries.

Methods: Diabetes was induced by i.p. injection of 55 mg/kg streptozotocin (STZ) to rats on 2nd day of pregnancy (n = 23). Control rats were injected with citrate buffer (CB, n = 21). Second order radial arteries feeding the placenta were dissected from 20 day pregnant rats, cannulated and pressurized in an arteriograph. Phenylephrine (PE) was applied to pre-constrict vessels, and ACh was tested in increasing concentrations in the presence of L-NNA and indomethacin. Some arteries were pre-treated with either 1 μM of PDBu or bisindolylmaleimide (BIS) to activate or inhibit PKC. Fura-2 based SMC or EC $[Ca^{2+}]_i$ responses were studied as well. Levels of phosphorylated PKC were determined in uterine arteries of STZ vs. CB rats by Western blot analysis.

Results: Diabetes resulted in reduction of EDHF-mediated vasodilation from 90 ± 4 % (n = 6) to 26 ± 9 % (n = 5) at 10 μM ACh. SMC and EC $[Ca^{2+}]_i$ responses were markedly reduced as well. PDBu inhibited ACh-evoked vasodilation (14 ± 5 %) and associated SMC $[Ca^{2+}]_i$ responses of CB vessels (n = 7). Exposure of STZ arteries to BIS partially restored EDHF-mediated vasodilation (to 72 ± 10 %, n = 5) and SMC $[Ca^{2+}]_i$. PDBu reduced EC $[Ca^{2+}]_i$ responses of CB vessels to 0.3 μM ACh by 50 ± 7 % (n = 6). BIS significantly improved EC $[Ca^{2+}]_i$ elevations to ACh in STZ arteries. Diabetic pregnancy was associated with increased activity of conventional PKC isoforms in uterine vasculature.

Conclusion: Impaired EDHF-mediated uterine vasodilation in diabetic pregnancy is associated with enhanced PKC activity and is due to reduced EC Ca^{2+} signaling. Inhibition of PKC restores Ca^{2+} signaling and vasodilation and may serve as a therapeutic strategy to improve uterine endothelial function in pregnancies complicated with diabetes.

Supported by NIH HL088245

O-116

DNA Hypomethylation in Neutrophils Is Associated with Increased Expression of Thromboxane Synthase in Preeclampsia. Ahmad A Mousa, Sonya L Washington, Jerome F Strauss III, Scott W Walsh. *OB-GYN, Physiology & Biophysics, Virginia Commonwealth University Medical Center, Richmond, VA, USA.*

Introduction: We recently discovered that thromboxane synthase (TBXAS1), an enzyme that generates a potent lipidic vasoconstrictor and platelet activator, thromboxane A2, is increased in omental fat arteries of preeclamptic women as compared to normal pregnant women. Increased TBXAS1 protein levels were present in endothelial cells, vascular smooth muscle cells, and especially infiltrating neutrophils, which strongly express TBXAS1. In this study, we determined the DNA methylation status of the *TBXAS*1 gene in omental arteries and determined if hypomethylation results in increased expression of TBXAS1 in a neutrophil-like cell line (HL-60).

Methods: DNA was extracted from omental fat arteries obtained from 5 normal pregnant and 7 preeclamptic women. DNA was bisulfite treated and analyzed for DNA methylation using the Illumina HumanMethylation27 BeadChip. HL-60 cells were treated with 10 μM 5-aza-2-deoxycytidine (5-Aza), an inhibitor of DNA methylation, for 48 h, 0.01 μM phorbol myristate acetate (PMA), an activator of protein kinase C, for 24 h or 5-Aza + PMA. TBXAS1 expression was assessed by qRT-PCR and Western blotting.

Results: *TBXAS1* was significantly hypomethylated in preeclamptic omental arteries ($\Delta\beta$=-0.24, $p< 0.001$). Treatment of HL-60 cells with 5-Aza resulted in significant increase in *TBXAS1* expression as compared to controls (2.6 ± 0.2-fold, $p<0.001$). PMA alone did not increase *TBXAS1*, but when combined with 5-Aza resulted in significant increase in *TBXAS1* as compared to controls (3.8 ± 0.4-fold, $p<0.001$), PMA alone ($p<0.001$) or 5-Aza alone ($p<0.001$). Western blotting confirmed increased TBXAS1 expression.

Conclusion: The *TBXAS1* gene is significantly hypomethylated in omental arteries of preeclamptic women, and experimentally induced hypomethylation increases its expression in a neutrophil-like cell line.

Speculation: Epigenetic mechanisms (DNA hypomethylation) in neutrophils contribute to the increased expression of TBXAS1 in systemic blood vessels of preeclamptic women, which may contribute to the hypertension and hematologic abnormalities associated with this syndrome. NIH HL069851, P60MD002256

O-117

Sprouty4 Selectively Binds and Regulates the Function of Endothelial Nitric Oxide Synthase Localized on Plasma Membrane and *trans*-golgi Complex. Quan Luo, Lin Feng, Dong-bao Chen. *Dept of Ob/Gyn, Univ. of CA, Irvine, CA, USA.*

Introduction: Endothelial nitric oxide (NO) synthase (eNOS) synthesizes NO; however, we have recently shown that when binds to the receptor tyrosine kinase antagonist sprouty, eNOS function is shifted to produce

superoxide. The eNOS is a highly-trafficking protein that localizes in various organelles, including Golgi, mitochondria, plasma membrane and nucleus, and its function is closely related to its proper subcellular localization is he eNOS function. However, it is unknown if sprouty selectively interacts with organelle-restricted eNOS. **Objective**: to test a hypothesis that sprouty interacts with eNOS in an organelle-restricted fashion to regulate NO production. **Methods**: Mammalian expression plasmids carrying wild type human eNOS (WT-eNOS), mitochondria-localized eNOS (Mito-eNOS), *cis*-golgi-localized eNOS (S17-eNOS) plasmid, the *trans*-golgi-localized eNOS (GRIP-eNOS), plasma-membrane-localized eNOS (CAAX-eNOS), or nucleus-localized eNOS (NLS-eNOS), was co-transfected with flag-tagged Spry4 in HEK 293T cells using GenJet™ transfection reagent. Forty hours post-transfection, total cell lysates were prepared for co-immunoprecipitation of eNOS and sprouty4 using anti-eNOS and anti-flag antibodies. Cellular distribution and co-localization of flag-Spry4 and organelle-restricted eNOS were assessed by using double immunofluorescence confocal microscopy. NO production was measured by using DAF-2DA imaging. **Results**: Sprouty4 co-immunoprecipitates selectively with wild-type eNOS, *trans*-golgi and plasma membrane restricted eNOS, which were confirmed by double immunofluorescence confocal microscopy. Ca2+ ionophore A23187 significantly stimulated NO production in cells transfected with wild-type, *trans*-golgi and plasma membrane localized eNOS, which was abolished by co-transfection with sprouty4. **Conclusion:** Sprouty selectively binds to organelle-restricted eNOS to regulate NO production (Supported by RO1 HL70562 and R21 HL98746).

O-118

Electrical Properties of P-UAEC and the Role of Agonist Induced Hyperpolarization in Sustained Ca2+ Signaling. Roxanne Alvarez, Fu-Xian Yi, Bikash Pattnaik, Ian Bird. *Dept ObGyn and Pediatrics, Univ of Wisconsin Madison, WI.*

Uterine artery endothelial cells from pregnant ewes (P-UAEC) typically show sustained Ca2+ bursting in response to ATP stimulation due to periodic activation of TRPC channels. This in turn depends upon gap junction function, and the resulting bursts facilitate production of the vasodilator nitric oxide. Cells from nonpregnant ewes (NP-UAEC) fail to show prolonged burst responses and also show poor gap junction coupling. TRPC3 function is known to be sensitive to cell membrane potential, and may be enhanced as the result of cell-cell communication. We propose improved gap junction communication during pregnancy allows electrical coupling that shifts cells towards a membrane potential range supportive of TRPC3 activity necessary for sustained Ca2+ bursting. ***Objective:*** Our goal was to establish basic UAEC electrical properties and specifically the cell's resting membrane potential. Also, to then further determine possible changes in membrane potential in response to ATP stimulation, and to establish if a direct coupling relationship exists between Ca2+ bursting and the cell's membrane potential. ***Methods:*** Ovine P-UAEC (passage 4) were grown in 35 mm glass bottom dishes to <20% density for electrophysiology or 100% for use in dye imaging. Whole-cell patch clamping was performed on isolated, voltage clamped cells. Cells were loaded with Fura-2 (Ca2+ dye) followed by DIBAC4 (membrane potential dye). Simultaneous imaging of [Ca2+]i and membrane potential was acquired for 35 minutes under basal or ATP (100uM) stimulation. Data from stimulated cells were then compared to vehicle control cells. ***Results:*** Resting membrane potential of P-UAEC is negative 20mV ±2.0, capacitance is 14pF ±1.7, and resistance is 4.0GΩ ±0.54. ATP (100uM) stimulation of P-UAEC results in an agonist specific and progressive membrane hyperpolarization that continues for as long as sustained Ca2+ bursts occur. The approximate drop in membrane potential over 30 minutes is a further 20 mV. ***Conclusion:*** We have established the basic electrical properties in P-UAEC and that ATP stimulation does indeed result in further membrane potential hyperpolarization during Ca2+ bursting. We are currently extending these studies in NP-UAEC. Further studies are needed to determine the cause and effect relationship between changes in membrane potential and Ca2+ bursting in P- and NP-UAEC and the dependence of these responses on gap junctions. Funded by NIH R01 HL079020.

O-119

Vasodilator Role of Hydrogen Sulphide (H_2S) in Human Placentae from Healthy and Pathological Pregnancies. T Cindrova-Davies,[1] EA Herrera,[1,2] YG Niu,[1] DA Giussani,[1] GJ Burton. *[1]PDN, University of Cambridge, United Kingdom; [2]Facultad de Medicina, Universidad de Chile, Chile.*

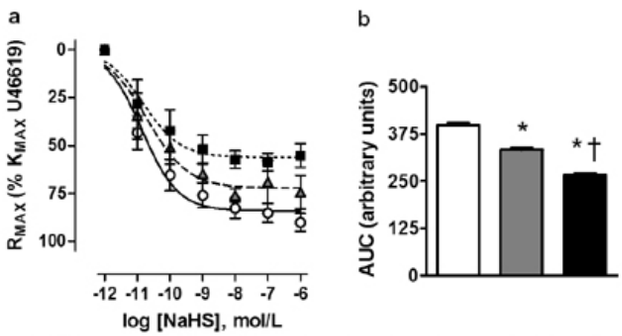
Background: Hydrogen sulphide (H_2S) has joined NO and CO as the third novel gaseous vasodilator. Endogenous H_2S synthesis is regulated by cystathione γ-lyase (CSE) and cystathione β-synthase (CBS). H_2S-induced targeting of K_{ATP} channels hyperpolarises cell membranes and thus relaxes smooth muscle cells, inducing vasodilatation. However, the haemodynamic role of H_2S in the fetoplacental circulation is unknown.

Methods: Single lobes of 8 caesarean-delivered human placentae were perfused with equilibrated (95%O_2/5%CO_2, pH7.4) Earle's bicarbonate buffer (containing dextran, albumin,L-arginine,heparin) by cannulating the chorionic artery and vein of the lobe. Perfusion pressure and flow were measured. Following pre-constriction with a thromboxane mimetic U46619 (10^{-7}mol/L), increasing doses of H_2S donor NaHS (10^{-12}to10^{-6}mol/L) were infused. During the experiments, L-NAME (10^{-5} mol/L) and glibenclamide (10^{-5}mol/L) were administered sequentially to inhibit endogenous NO synthesis and to block K_{ATP} channels, respectively. In addition, the RNA and protein expression of CBS and CSE were determined in caesarean-delivered placentae from 6 healthy, 6 pre-eclamptic and 6 IUGR human pregnancies. CBS and CSE were immunolocalised in placental tissues.

Results: H_2S led to significant concentration-dependent reductions in chorionic vascular resistance (Fig.1). The vasodilator effect of H_2S (AUC arbitrary units: 397±7) was significantly diminished by L-NAME (332±6) and further reduced by glibenclamide (266±5;P<0.05). CBS and CSE were localised in the placental syncytiotrophoblast and vascular smooth muscle cells, respectively. CSE RNA and protein levels were significantly down-regulated in pre-eclamptic (protein 40.5%, RNA 0.44) and IUGR (43%, 0.44) compared to healthy pregnancy (100%, 1; P<0.05).

Conclusions: We show for the first time that H_2S is a potent vasodilator in the human placenta and that CSE enzyme responsible for its synthesis is down-regulated in pregnancies associated with impaired placental blood flow and fetal growth restriction.

Supported by the Wellcome Trust



Vasodilator response to NaHS in human placentae. Values are the mean ± S.E.M for the concentration-response curves (A) and of the area under the curve (B, AUC) to sodium hydrosulphide (NaHS). Groups are untreated placentae (white circle/histogram, n=6), placentae treated with the nitric oxide synthase blockade, L-NAME (grey triangle/histogram, n=6), and placentae treated with the K_{ATP} blockade, glibenclamide (black square/histogram, n = 6). Significant differences (P<0.05) are: * vs untreated group, † vs LNAME group (One-Way ANOVA + Student Newman-Keuls Test).

O-120

A Role for Plasma Protein Glycosylation in Endothelial Cell Damage by Inflammatory Mediators in Preeclampsia. Shannon K Flood-Nichols,[1] Peter G Napolitano,[1] Danielle L Ippolito.[2] *[1]Division of Maternal Fetal Medicine, Madigan Healthcare System, Tacoma, WA, USA; [2]Department of Clinical Investigation, Madigan Healthcare System, Tacoma, WA, USA.*

Introduction: The pathogenesis of preeclampsia begins with abnormal placentation, ultimately resulting in systemic maternal vascular endothelial cell damage. Dysfunctional endothelial cells recruit systemic inflammatory mediators and triglycerides, leading to the clinical features of preeclampsia such as proteinuria and hypertension.

Objective/Hypothesis: We hypothesize that abnormal plasma protein glycosylation patterns lead to endothelial dysfunction in preeclampsia by enhancing recruitment of inflammatory mediators to vascular endothelium. The objective of this study was to determine the role of plasma O-linked glycosylation in leukocyte adhesion in normal and preeclamptic pregnancies.

Methods: Maternal plasma was prospectively collected from women later diagnosed with preeclampsia or normotensive pregnancies. First, second, or third trimester plasma from n=5 women per cohort was treated with enzymes to remove sialic acids and O-linked glycoprotein linkages. Untreated control plasma or enzyme-treated plasma was used to condition fluorescently labeled monocyte cell lines *in vitro*. Adhesion of conditioned monocytes to umbilical cord vein endothelial cell monolayers was quantified by microscopy and spectrophotometry.

Friday

Results: Monocytes conditioned with normotensive plasma adhered less to endothelial monolayers (65-70% of no plasma vehicle controls) in all trimesters ($p<0.05$ by paired students t-test). However, pre-conditioning with preeclampsia plasma showed no attenuation of monocyte adhesion relative to vehicle treated controls in trimesters 1 and 2 ($p>0.05$), but conditioning monocytes with third trimester preeclampsia plasma reduced monocyte adhesion to 75% vehicle treated controls. Removal of O-linked glycosylation and sialic acids reversed the attenuation ($p<0.05$ by paired students t test).
Conclusions: These results suggest a novel role for plasma glycoprotein interaction with monocytes and endothelial cells in pregnancies complicated by preeclampsia. Glycoproteins may represent therapeutic targets for early intervention in ameliorating the endothelial dysfunction associated with preeclampsia.

O-121

Impaired DNA Repair: A Novel Explanation for Oocyte Aging. Kutluk Oktay,[1,2] Shiny Titus,[1] Fang Li,[1] Volkan Baltaci,[1] Sumanta Goswami.[1,3] *[1]OB-GYN/Cell Biology & Anatomy, NY Medical College, Valhalla, NY, USA; [2]Biology, Yeshiva Univ., New York, NY, USA.*
Objective: The molecular mechanisms behind oocyte aging are unknown. Recent work indicated that women with BRCA mutations may have low response to ovarian stimulation and experience menopause earlier. Because BRCA is a key double strand DNA break (DSB) repair gene, we hypothesized that impaired DSB repair and resulting accumulation of DSBs cause oocyte reserve depletion.
Materials and Methods: Given the age-related decline in oocyte yield after ovarian stimulation (41±15 at 4 wks to 11±5 at 9-mo), we used "young" (4-wk-old) and "old" (9-mo-old) female FVB mice (n=24). Primordial follicles (PDF) were assessed for DSBs by γH2AX IHC in ovarian sections. GV oocytes were assessed by confocal microscopic quantification of γH2AX foci. The expression of ATM-mediated DSB repair pathway genes were analyzed by qRT-PCR in PDF captured by laser dissection (LD) as well as single GV oocytes. The same was also analyzed in single human oocytes (n=20/age group) by QRT-PCR from young (age<27) and old (age>35) subjects. Ovarian reserve was assessed by serum AMH in women with (n=8, age=34±2.3) vs. without BRCA1 mutations (n=30, age=37±0.8).
Results: γH2AX-positive PDF increased significantly in old mice compared to young (16±3 vs. 10±2; p=0.002). Mean number of γH2AX foci was also significantly higher in GV oocytes of old vs. young (1,279±594 vs. 373±258; p=0.01). By QRT-PCR from single mouse GV oocytes, the expression of MRE11, ATM, BRCA1, genes involved in sensing DSBs and activating repair, were downregulated by 50-99% with age. In PDF from old mice, the expression of BRCA1 was downregulated by 77-89%. Consistent with findings in rodent oocytes, qRT-PCR of single human oocytes showed that the key genes in the DSB repair pathway were down-regulated with age. Strikingly, BRCA1 expression showed an inverse correlation with age, and women with BRCA1 mutations had significantly lower AMH levels compared to those without (1.3±0.3 ng/ml vs. 2.5±0.3, ANCOVA adjusting for age, $p<0.05$).
Conclusions: These translational data support our novel hypothesis that oocyte aging is associated with accumulation of detrimental DSBs owing to the impairment of DSB repair in the aging oocyte. It appears that BRCA1 gene expression is a strong indicator of oocyte age, and DNA repair is vital for oocyte health. These findings can be paradigm-shifting in understanding oocyte aging.
Support: RO1 HD053112

O-122

Microscopic Study of Component-Dependent Tissue Elasticity in Vaginal Connective Tissues. William W Kobak,[1] Rong Wang,[2] Jacob Rotmensch.[3] *[1]Department of Obstetric and Gynecology, University of Illinois, Chicago, IL; [2]Department of Chemistry, Illinois Institute of Technology, Chicago, IL; [3]Rush University Medical Center, , Chicago, IL.*
Conditions such as urinary incontinence, prolapse and sexual dysfunction have age-related components. This study was designed to ascertain the precise biochemical mechanism responsible for age-related changes in vaginal tissue. Atomic force microscopy (AFM) has emerged as a powerful tool for the nanoscale characterization of biological surfaces before clinical changes become apparent.
Methods:
Fresh samples of vaginal epithelium from 18 pre-menopausal subjects and 20 post-menopausal subjects were harvested and sectioned using a vibratome to 10 um. One of the two adjacent slides was used for Gomori staining (fixed) in order to confirm the tissue type being analyzed, and the other slide (fresh) was used for AFM study. With the power of imaging individual collagen fibers at high resolution, we performed elasticity mapping at small scales to evaluate the elasticity of local regions.
Results:
The mean age of the pre-menopausal subjects was 38 years (22-52) and 59 years (49-73) for the post-menopausal subjects. An average of 200 measurements were obtained at 2-3 different areas of each sample. Elasticity measurements were obtained for each area sampled and averaged. Elasticity measurements were obtained for both collagen and muscle in pre- and post-menopausal samples. We tested differences in elasticity using Wilcoxon signed rank and student t-tests. There was a significant change in tissue elasticity measurements for post-menopausal vaginal muscle and for post-menopausal collagen.

Changes in elasticity by menopause

Group	Elasticity Mean in kilopascals	P value
Collagen		p<0.0001
Pre-menopause	6000(3500-8000)	
Post-menopause	4500(1500-10,000)	
Muscle		p<0.0001
Pre-menopause	6500(4500-10,000))	
Post-menpause	8000(5500-10,000)	

Conclusion:
Age-related changes in component tissues of vaginal epithelium are detectable at the nanoscopic level. These changes demonstrate a consistent differential response to ageing. The elasticity of vaginal collagen decreases with menopause, while muscle becomes stiffer. Studies are currently in progress to determine if this technique can be applied to the study of other conditions where ageing connective tissue may be linked to the development pelvic organ prolapse.

O-123

Antiphospholipid Antibodies Induce Trophoblast IL-1β Production through the Nalp3/ASC Inflammasome. Melissa J Mulla,[1] Jan J Brosens,[2] Jane E Salmon,[3] Larry W Chamley,[4] Vikki M Abrahams.[1] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University; [2]Division of Reproductive Health, University of Warwick; [3]Hospital for Special Surgery, Weill Cornell Medical College; [4]Department of Obstetrics and Gynecology, University of Auckland.*
Objective: Women with antiphospholipid syndrome (APS) are at risk for recurrent pregnancy loss and late pregnancy complications, like preeclampsia. Antiphospholipid antibodies (aPL) directly target the placenta by binding beta2-glycoprotein I (β2GPI) expressed by the trophoblast. We recently demonstrated that anti-β2GPI Abs induce secretion of pro-inflammatory IL-1β by human first trimester trophoblast. IL-1β secretion occurs after pro-IL-1β is processed into its active form. This is mediated by the inflammasome, a complex of Nalp3 and apoptosis-associated speck-like protein containing a CARD (ASC) that subsequently activates caspase-1, leading to IL-1β processing. Therefore, the objective of this study was to determine if aPL induces trophoblast IL-1β production via the inflammasome.
Methods: The human first trimester trophoblast cell line, Sw.71, was incubated with or without a mouse anti-human ββ2GPI mAb, IIC5 (20μg/ml), or human aPL-IgG (500μg/ml) for 72hrs. Mouse IgG1 or human IgG served as controls. IL-1β secretion and processing were determined by ELISA and Western blot, respectively. Caspase-1 activation was determined by Western blot and was blocked using a specific inhibitor. Nalp3 and ASC knockdown in Sw.71 cells was achieved using lentiviral transfection of specific shRNAs.
Results: Following a 72hr incubation, both IIC5 and human aPL-IgG significantly upregulated trophoblast IL-1β secretion when compared to the controls ($n=5$; $p<0.05$). This correlated with increased protein expression levels of active IL-1β and active caspase-1. Caspase-1 inhibition significantly reduced IIC5-induced IL-1β secretion by 30.5±12.0% in the Sw.71 cells ($n=4$; $p<0.001$). Similarly, Nalp3 and ASC knockdown significantly reduced the IIC5-induced IL-1β secretion by 46.5±11.5% and 54.5±6.2%, respectively ($n=4$; $p<0.001$).
Conclusion: These findings demonstrate that aPL activates the Nalp3/ASC inflammasome in the trophoblast, leading to IL-1β processing and secretion. This may provide a novel mechanism for the induction of inflammation at the maternal-fetal interface leading to placental dysfunction and adverse pregnancy outcome in patients with APS.
This work was funded by the American Heart Association.

O-124

Granulocyte Colony Stimulating Factor Maintains Ovarian Follicle Numbers in Mice Treated with High-Dose Chemotherapy by Decreasing Chemotherapy-Related Ischemia. Malgorzata E Skaznik-Wikiel, Megan McGuire, Meena Sukhwani, Thomas C Krivak, Alexandar Rajkovic, Kyle Orwig. *Dept. of OB/GYN and Reproductive Sciences, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA.*

Objective: Ovaries are very sensitive to cytotoxic treatment. Cyclophosphamide (CTX) and busulfan (BUS) are agents commonly implicated in causing damage to oocytes in a dose-dependent manner. Current fertility preservation options are limited, and there is a need to find new strategies attenuating gonadotoxicity. The goal of our study was to determine if granulocyte colony stimulating factor (G-CSF) alone or in conjunction with stem cell factor (SCF) preserves follicle numbers in animals treated with high-dose chemotherapy.

Methods: 6-week-old female mice were assigned to one of 4 treatment groups (n=13 per group). Group I received one-time injection of CTX (100mg/kg; IP) and BUS (12mg/kg; IP). Group II received G-CSF (50 µg/kg/d; IP) and SCF (100 µg/kg/d; IP) for 5 days with CTX and BUS on the third day. Group III received G-CSF alone for 5 days with CTX and BUS on the third day. Group IV (vehicle control) received normal saline for 5 days with 50% DMSO on the third day. Ovaries were collected on day 2 and 21 after chemo treatment for assessment of primordial, primary, and secondary follicle counts, and on day 21 for assessment of microvessel density (CD31 IHC).

Results: In mice that were sacrificed after 2 days there were no differences between groups in the number of primordial, primary, and secondary follicles. However when mice were sacrificed on day 21, ovarian follicles were nearly depleated (100/ovary; $p<0.0001$) in chemo treated mice relative to vehicle treated controls (2160/ovary). Mice injected with chemo and G-CSF/SCF (445/ovary), as well as with G-CSF alone (350/ovary), had a significantly larger pool of total follicles compared with mice treated with chemo alone ($p<0.0001$). G-CSF/SCF (68 vessels/mm^3) and G-CSF alone (61 vessels/mm^3) treated animals also had a higher microvessel density than chemo-only (32 / mm^3) treated animals 21 days after treatment. The numbers of vessels in these two groups were comparable to vehicle treated controls (60/mm^3).

Conclusions: Treatment of mice with G-CSF/SCF or G-CSF alone protects the ovarian follicle pool from the damaging effects of high-dose chemotherapy. A potential mechanism of this protection would include a decrease in chemo-related ischemia. Future studies should focus on functional assessment of preserved follicles.

O-125

Use of Pregnancy Enhanced T Regulatory Cells To Treat Autoimmunity. Sonal N Patel, Daniel A Kahn. *Obstetrics and Gynecology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA.*

Many autoimmune conditions ameliorate during pregnancy. T regulatory (Treg) cellular response is induced during pregnancy in response to specific fetal antigens. Functional Tregs are required for a successful pregnancy. We explored the transfer of Treg mediated immune tolerance as a treatment for experimental allergic encephalomyelitis (EAE).

Methods

Tregs were isolated by FACS from splenocytes taken from 8-week-old C57BL/6-FoxP3-GFP females on day 18.5 post-vaginal plug from a syngeneic mating or from virgin non-pregnant mice. Separately, EAE was induced with Myelin Oligodendrocyte Glycoprotein (MOG35-55)/CFA in male and virgin female 6-week-old C57BL/6 mice. On day 5, sorted Tregs (100,000/mouse) were transferred i.p. from either pregnant or non-pregnant mice. In a separate experiments, female mice were treated with 10,000 sorted Tregs from virgin or 18.5 post plug female C57BL/6-FoxP3-GFP mice.

Results

Males had a more severe disease course than females. In males, Tregs from non-pregnant mice resulted in a small amelioration of disease. In females, the non-pregnant Tregs didn't result in any improvement. In contrast, males treated with pregnancy induced Tregs had dramatically less disease severity and incidence. Females treated pregnancy induced Tregs never showed signs of disease when treated with 100,000 Tregs. When treated with as few as 10,000 pregnancy enhanced Tregs, female C57BL/6 induced to have EAE show less incidence and severity of disease.

	Disease Incidence				
	Untreated	Nonpregnant Treg	Pregnancy Treg (100,000)	Nonpregnant Treg (10,000)	Pregnancy Treg (10,000)
Male	8/8	6/7	4/8	4/4	4/4
Female	7/8	6/6	0/8	4/4	1/4

Mice with clinical scores > / 1.0

Conclusions

Pregnancy immune tolerance is transferable through Tregs. Pregnancy leads to a dramatic enhancement in Treg functionality that is capable of arresting an evolving immune response. Further exploration of pregnancy induced changes in Treg function may lead to novel treatments for autoimmune disease and deepen our understanding of immune tolerance during pregnancy.

Research support: Joint Center for Translational Medicine and UCLA Scholars for Translational Medicine.

O-126

Maternal Protein Restriction (MPR) during Pregnancy Leads to Alteration in Oxidative Stress in the Testis of Male Offspring (OFF). Guadelupe L Rodriguez-Gonzalez,[1] Luis Reyes,[1] Claudia Vega,[1] Omar Saldana-Ahuactzi,[1] Fernando Larrea,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] *[1]Reproductive Biology, Instituto Nacional de Ciencias Medicas y Nutricion SZ, Mexico City, Mexico; [2]OB/GYN, Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*

Objective. The onset of many diseases, including reproductive dysfunctions are programmed in utero or the early postnatal period[1]. We have shown that MPR in pregnancy impairs male reproductive capacity[2]. It is suggested that early life nutritional adversity is associated with increased oxidative stress (OS)[3]. OS is implicated in t pathogeneses of many diseases and developmental defects including male infertility[4]. We therefore investigated effects of MPR during pregnancy on testicular OS and its impact on the germ cell (GC) number, during the first wave of spermatogenesis.

Methods. We studied male rat OFF of mothers fed either control (C) (20% casein) or restricted (R) (10% casein) isocaloric diet during pregnancy. After birth all rats were fed with C diet. At 19 days of gestation (dG) and at postnatal day (PND) 21 and 36, male OFF were euthanized and testis were removed, one frozen to evaluate t lipid peroxide level using a thiobarbituric acid reactive substances assay, which monitors MDA (malonaldialdehyde) production; and other fixed to evaluate GC number. Data M ± SEM; analysis by t-test; n=5, $p<0.05$.

Results. At 19dG we did not find differences in MDA concentration, however at PND 21 and 36 MDA levels were statistically higher in the R group vs C. The total number of GC were significantly lower in the R group at 14 and 21 PND.

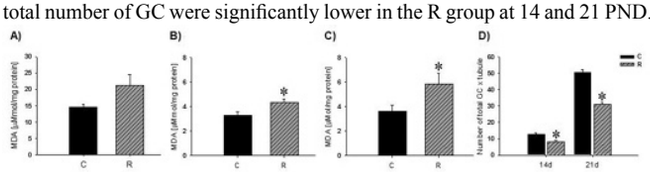


Fig 1. A, B and C: levels of MDA from homogenates of testis at 19dG, 21 and 36 PND respectively; D: total number of germ cells at 14 and 21 PND. Mean ± SEM, n=5, $p<0.05$ * vs C.

Conclusions. In the prepuberal testis, MPR during pregnancy leads to oxidative damage and reduction in the number of GC. These processes may constitute the underlying mechanisms that decrease fertility rate in the R group OFF.

References. 1) Int J Androl. 2010;33:394-404, 2) J Physiol. 2005;563:275-84, 3) PLoS One. 2010;5:e15558, 4) Dev Growth Differ. 2010;52:657-63.

O-127

In Vitro Activation of Cryopreserved Ovarian Cortex Follicles from Oncologic Patients. Sonia Herraiz,[1] Patricia Torres,[2] Cesar Diaz,[2] Patrocinio Polo,[2] Jose M Rubio,[2] Antonio Pellicer,[1,2] Edurne Novella-Maestre.[2] *[1]Research, Fundación IVI-IUIVI; [2]Gynecology, Hospital Universitario La Fe.*

Cryopreservation of ovarian cortex (COC) and subsequent orthotopic transplantation (OT) is an available option for Fertility Preservation (FP) in young oncologic patients. However freezing/thawing protocols lead to a significant loss of follicular population in tissue. During reproductive life, only some follicles are activated for development. Employing an oocyte-specific deletion of PTEN gene involved in oocyte maturation, premature activation of primordial dormant follicles was recently induced. The aim of the study was to apply this method of enhancement of primordial dormant follicles in COC from oncologic patients, with a PTEN inhibitor, to counteract the lack of follicles associated to the cryopreservation techniques in order to gain efficiency in FP. METHODS The study included 10 cortical ovarian biopsies from women undergoing FP with cancer diagnosis. Cryopreserved/thawed (CA n=4) and fresh (FA n=6) biopsies (3mm^3) were treated for 1h with 100µM bpV(pic), a PTEN inhibitor, in α-MEM at 37ºC and 5%CO2 followed by 24h with the same media supplemented with 0.3UI/mL FSH. Cryopreserved/thawed (C) and fresh (F) biopsies incubated without inhibitor were used as controls. Follicular

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activation was evaluated by immunohistochemical study of nuclear extrusion of Foxo3 and antimüllerian hormone (AMH) expression. TUNEL assay and follicular counting were developed to study cell damage.
RESULTS A 2-fold increase of Foxo3 nuclear extrusion was found in cryopreserved biopsies (CA:48.8±10.3% vs. C:22.9±15.7%, p=ns). AMH expression was decreased by cryopreservation but activation protocol restores AMH levels (F:36.1±12.4% vs. C:25.7±9.0% vs. CA:33.0±13.1%).
In fresh samples Foxo3 (FA:59.3±5.4% vs. F:21.5±9.9%) and AMH expression (FA:39.3±12.1% vs. F:29.8±6.1%) were significantly enhanced. Despite of no differences were found in follicle counting between groups an increase of TUNEL positive stromal cells were found in FA and CA.
CONCLUSION Findings showed that the use of PTEN inhibitor was effective to induce activation of dormant follicles in fresh tissue at lower dose than previously has been reported. Also results demonstrated that the activation was also enhanced when inhibitor was applied to COC suggesting that the use of this procedure, previously to COC-OT could allow the generation of a large supply of germ cells to improve effectiveness of FP methods.

O-128

Testosterone Affects Normal Maternal Vascular Adaptations to Pregnancy by Impairing Endothelial NO-Mediated Vasodilation. Vijayakumar Chinnathambi, Chandra Yallampalli, K Sathishkumar. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
The enhanced release of the endothelium-derived vasodilators is critical and precedes other vascular adaptations that occur during pregnancy. Among many factors, sex steroid hormones including estradiol and progesterone play an important role in these vascular adaptations. However, little is known about the role of androgens. Plasma testosterone levels are elevated in preeclampsia, PCOS mothers and pregnant African-American women, who often tend to develop gestational hypertension. We tested whether testosterone affects the normal vascular adaptations to pregnancy by impairing endothelium-dependent vasodilation and causing hypertension in pregnant rats.
Methods: We injected pregnant Sprague-Dawley rats with vehicle or testosterone propionate (TP; 0.5 mg/Kg/day from gestation day (GD) 15-19) to increase plasma testosterone level two-fold, similar to that observed in clinical conditions like preeclampsia. Blood pressure and mesenteric vascular reactivity were assessed using telemetry and wire myograph, respectively. The involvement of NO, prostaglandins, and EDHF was assessed using specific pharmacological inhibitors.
Results: Fetal and placental weights were significantly reduced in TP group. Mean arterial pressure was similar between control and TP dams at early phase of TP treatment; however, after GD 19, blood pressure in TP dams were significantly higher compared with controls. The heart rate was not significantly different between controls and TP dams. Endothelium-mediated relaxation responses to acetylcholine were significantly impaired in mesenteric vasculature of TP dams (Control: $logEC_{50}$ = -7.38.04; E_{max} = 99.90.97; TP: $logEC_{50}$= -7.05.06; E_{max} = 89.41.89). NO-mediated vasodilatation was significantly reduced in TP mesenteric arteries (E_{max} = 45.45.48) compared to controls (E_{max} = 76.495.06). EDHF- and prostaglandin-mediated vasodilation was maintained in TP mesenteric arteries. Vasodilation to sodium nitroprusside was similar between control and TP mesenteric arteries.
Conclusion: Elevated maternal testosterone, at concentrations relevant to those observed in abnormal clinical conditions, causes hypertension with impaired maternal vascular adaptations, specifically blunting NO-mediated vasodilation. These results suggest a role for testosterone as one possible mediator of the increased vascular resistance associated with pregnancy-induced hypertension.

O-129

Progressive Decline of Oogonial Stem Cell Activity Contributes to Ovarian Aging. Ning Wang, Chonthicha Satirapod, Jonathan L Tilly. *Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.*
Background: mounting evidence indicates that female germline or oogonial stem cells (OSCs) exist in adult mammalian ovaries [Nature 2004 428:145; Biol Reprod 2009 80:2] and can generate functional oocytes [Nat Cell Biol 2009 11:631]. It remains unknown, however, if dysfunction of OSCs and a resultant impairment of oocyte renewal at some point during adulthood contributes to ultimate exhaustion of the ovarian reserve of primordial oocyte-containing follicles as females age.
Objective: to determine if OSC-based maintenance of primordial follicle numbers in mice during adulthood is negatively affected by the aging process.
Methods: Stra8 is a germ cell-specific gene transiently expressed during meiotic entry. We developed a suicide gene transgenic mouse line in which Stra8-expressing (differentiating) OSC progeny (Stra8+ OSCPs) can be reversibly targeted for ablation in vivo without directly affecting OSCs or oocytes since these cells lack Stra8. Accordingly, the extent by which the size of the primordial follicle reserve changes during ablation (loss due to an absence of new input) and post-ablation (regeneration from OSCs) periods was used to quantitate OSC activity in female mice at young (2-3 months), mid- (5-6 months) and late (10-11 months) reproductive ages.
Results: in young adult mice, ablation of Stra8+ OSCPs for 21 days resulted in a significant reduction in primordial follicle numbers from 3250 ± 296 to 1733 ± 283 per ovary ($P<0.05$). Once ablation was ceased, the primordial follicle pool was progressively rebuilt over the subsequent 21 days back to pretreatment levels (3,315 ± 289 per ovary). In middle aged mice, 21 days of ablation of Stra8+ OSCPs also caused a significant reduction in primordial follicle numbers (from 2151 ± 294 to 952 ± 225 per ovary; $P<0.05$). However, once ablation was ceased OSCs failed to fully regenerate the primordial follicle pool back to pre-treatment levels over the 21 day recovery period (1559 ± 357 per ovary), suggesting the onset of impaired OSC activity by this age. In female mice at late reproductive ages, ablation of Stra8+ OSCPs had no effect on the existing primordial follicle pool, suggesting a complete absence of OSC activity at this time.
Conclusion: OSC-mediated support of the ovarian reserve is progressively lost as females age and this likely contributes to exhaustion of the follicle pool during ovarian aging.
Support: NIH R37-AG012279, K99-AG039512.

O-130

Epigenetic Regulation of Endometrial Metabolism by KLF11 Via the Chromatin Silencing Complex Sin3a/Histone Deacetylase (HDAC). Zaid M Tabbaa, Ravi P Gada, Ye Zheng, Phoebe H Leonard, Raul Urrutia, Gwen A Lomberk, Gaurang S Daftary. *Epigenetics and Reproductive Disease Laboratory, Mayo Clinic, Rochester, MN, USA.*
Objective: Recent discoveries that the Krüppel-like transcription factors (KLF) regulate key metabolic signaling pathways including sex-steroids and xenobiotics could significantly impact human reproduction. Cytochromes p450 1A1 and 3A4 are key endometrial enzymes that metabolize estrogen as well as xenobiotics. Their metabolic fate determines estrogen bioavailability, and could affect environmental xenobiotic induced diseases such as endometriosis. Here we characterize the role of KLF11 in endometrial metabolism via histone deacetylase (HDAC) mediated chromatin remodeling.
Methods: KLF11 expression in Ishikawa and stromal cells was detected by PCR, western blot and immunofluorescence and in the endometrium by immunohistochemistry. Epigenetic cofactor-interaction was evaluated by immunofluorescence, co-immunoprecipitation and in vitro mutagenesis. Metabolic target genes were identified by microarray and confirmed by Chromatin Immunoprecipitation and PCR. Target-gene regulation was evaluated by PCR, EMSA and Luciferase Assay.
Results: KLF11 regulated key endometrial cytochrome p450 enzyme mediated metabolic pathways. We have identified the cytochrome p450 enzyme genes CYP1A1 and 3A4 as endometrial KLF11 targets; KLF11 bound their promoters and repressed mRNA expression levels ($p < 0.001$) as well as Luciferase promoter-reporter activity ($p < 0.01$). KLF11 co-localized with Sin3a and HDAC1 and 2. CYP1A1 and 3A4 repression was reversed by KLF11 and Sin3a siRNA as well as by a mutation in KLF11 that abrogated Sin3a binding. Target gene repression by KLF11 was therefore mediated via Sin3/HDAC binding, resulting in promoter histone deacetylation. KLF11-Sin3a localized predominantly to nuclear euchromatin domains indicating that KLF11 was involved in transient repression of actively expressed genes such as endometrial p450 enzymes. .
Conclusion: This is the first study to identify a role of the metabolic regulator KLF11 in endometrial cells implicating estrogen/xenobiotic metabolic pathways. Altered endometrial metabolites could potentially affect endometrial receptivity and disease such as endometriosis and cancer. KLF11 may have role in determining endometrial estrogen bioavailability and xenobiotic processing via novel, therapeutically reversible epigenetic mechanisms involving histone deacteylation.

O-131

Direct Reprogramming of Fibroblasts into Trophoblast Stem Cells. Matteo Moretto Zita,[1] Kristopher L Nazor,[3] Louise C Laurent,[2] Mana M Parast.[1] [1]*Pathology, University of California San Diego, La Jolla, CA, USA;* [2]*Reproductive Medicine, University of California San Diego, La Jolla, CA, USA;* [3]*Center for Regenerative Medicine, The Scripps Research Institute, La Jolla, CA, USA.*

Trophoblast, the epithelial compartment of the placenta, is the first lineage specified during mammalian embryonic development. Understanding this lineage specification, along with the processes involved in its further differentiation into cellular subtypes involved in uterine invasion and nutrient and gas exchange, is pivotal for determination of potential therapeutic approaches for prevention or treatment of pregnancy complications, including pregnancy loss, growth restriction, and preeclampsia. Early human trophoblast differentiation is hampered by the lack of an appropriate in vitro cell culture model: i.e. a "trophoblast stem" cell model, analogous to TS cells derived from mouse embryos. Recently, several groups have successfully reprogrammed fibroblasts into other somatic cell types by introduction of multiple genes, following the induced pluripotent stem ("iPS") cell concept. We have focused on the generation of induced trophoblast stem ("iTS") cells that will allow us to decipher the molecular mechanisms responsible for establishment of the trophoblast lineage. Since the derivation and culture of mouse TS cells have been well-established, we started by identifying a mouse TS cell-specific signature, by comparing the gene expression profiles of mouse TS, mouse embryonic stem cells (mESCs), and mouse embryo fibroblasts (MEFs), all from the same genetic background. We then selected a group of candidate genes, including CDX2 and Eomes, which we tested in a variety of combinations using a retroviral expression system for the ability to generate iTS cells from MEFs. The resulting cells showed a marked mesenchymal-to-epithelial transition, both based on morphology and expression of cytokeratin 7. In addition, compared to MEFs transduced with GFP-expressing retrovirus, the iTS cells showed enhanced expression of the trophoblast giant cell markers, Pl-I and Hand1. Taken together, these data suggest that the fibroblasts are at least partially reprogrammed toward the trophoblast lineage. This work presents proof of concept for direct reprogramming of fibroblasts towards an extraembryonic lineage, and could serve as a basis for establishment of such iTS cells for study of human trophoblast differentiation.

O-132

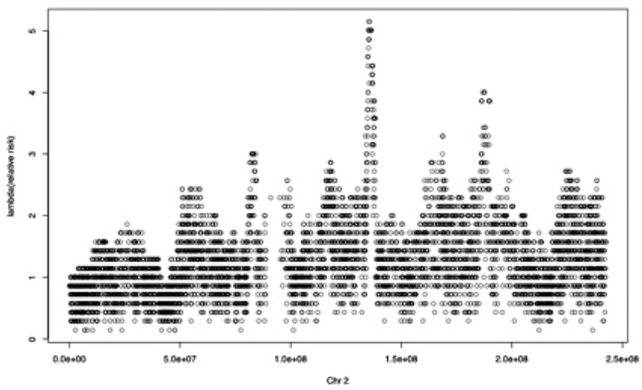
Shared Genomic Segment Analysis in Extended Families with Endometriosis. Kenneth Ward, Rakesh Chettier, Pam Farrington, Hans Albertsen. *Research, Juneau Biosciences, Salt Lake City, UT, USA.*

Endometriosis is a complex condition of uncertain etiology. Previous reports have shown familial clustering, but usually these studies have only considered close relatives. We have shown that excess relatedness is observed between endometriosis patients at genealogical distances corresponding to fourth cousins. The goal of this study is to use shared genomic segment analysis (SGSA) to confirm our prior observations and to identify specific chromosomal regions shared in excess by distant relatives in high-risk families.

Methods: Extensive family histories were obtained from over 1,000 women with surgically-confirmed endometriosis. We searched a genealogy database focused on the Western U.S. (GenDB), all available ancestors of the affected women. Probands also underwent DNA-based ancestry analysis using Affymetrix 6.0 SNP chips and the GERMLINE algorithm to confirm that relative pairs shared the expected number/length of genomic segments. Only affected women who were 5-10 meiosis apart (corresponding to first cousins once removed to fourth cousins) were considered. We scanned pairwise identity-by-descent (IBD) results across the genome to calculate relative risks for each 5 Mb genomic segment (pairs showing IBD/expected IBD).

Results: A total of 503 affected relative pairs were identified; this number of affected pairs was greater than expected ($p<0.001$). Several genomic regions showed excess sharing (sample data from chromosome 2 is shown in the Figure).

Conclusions: This study shows that endometriosis "runs in families" even at distant relationships. Since shared environment is significantly reduced among distantly related individuals, our data provide further evidence that genetic factors play an important role in endometriosis. SGSA in high-risk families is more powerful than and avoids many pitfalls of standard genome-wide association studies. The genomic IBD regions identified across extended families are likely to contain important genetic risk factors for endometriosis.

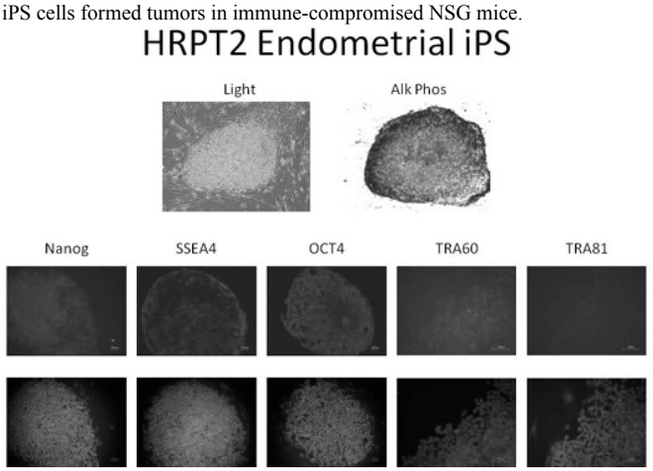


O-133

Disease-Specific Induced Pluripotent Stem (iPS) Cells Derived from the Endometrium of a Patient with *HRPT2* Germline Mutation. Erin Foran Wolff,[1,3] Chuanfeng Wu,[2] So Gun Hong,[2] Thomas Winker,[2] John Tisdale.[3] [1]*Unit on Reproductive and Regenerative Medicine, PRAE, NICHD, NIH, Bethesda, MD, USA;* [2]*Hematology Branch, NHLBI, NIH, Bethesda, MD, USA;* [3]*Molecular and Clinical Hematology Branch, NHLBI, NIH, Bethesda, MD, USA.*

Background: Familial Jaw Tumor-Hyperparathyroidism is a disorder characterized by sclerotic jaw tumors, hyperparathyroidism and renal cysts due to a mutation in the HRPT2 gene. This condition also exhibits a variety of atypical uterine tumors, recurrent pregnancy loss, and severe menorrhagia resulting in hysterectomy in the 3rd decade. Because of the pronounced reproductive phenotype of this mutation, the aim of this study was to develop an induced pluripotent stem cell line to be used as an in vitro model for gynecologic disease.

Methods/Results: Primary cell cultures were generated from affected polypous and nonpolypous endometrial tissue. Using forced expression of the reprogramming factors Oct4, Sox2, Klf4, and c-Myc via lentiviral (polycistronic, cre-excisable transgenes) gene transfer, we obtained several colonies resembling embryonic stem cell morphology. Individual colonies were picked and expanded for further characterization. All selected iPS cell lines stained positive for alkaline phosphates and expressed endogenous pluripotency markers (TRA 1-60, TRA 1-81, SSEA4, NANOG, OCT4). Finally, selected iPS cells formed tumors in immune-compromised NSG mice.



Conclusion: Here we describe the first reproductive disease specific iPS cell line, which was derived from a patient with a germline, single gene defect (HRPT2) with a marked reproductive phenotype. To the best of our knowledge, iPS cell lines have not been previously described from solid tumors or precancerous polyps, as we have demonstrated here. This novel HRPT2 iPS cell line could be an important new tool for studying reproductive tract disease.

Support: Intramural NIH: PRAE/NICHD and MCHB/NHLBI

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Expression and Epigenetic Regulation of Novel Tumor Suppressor EGF Containing Fibulin-Like Extracellular Matrix Protein 1 (EFEMP1) in Leiomyoma (LEIO) and Myometrium (MYO). Erica E Marsh, J Brandon Parker, Alanna Barrett, Ju Wu, Antonia Navarro, Serdar E Bulun. *Obstetrics and Gynecology, Feinberg School of Medicine - Northwestern University, Chicago, IL, USA.*

Background: Leiomyomata are highly prevalent benign smooth muscle tumors but their pathophysiology remains poorly understood. EFEMP1 has been shown to be a tumor suppressor and an antiangiogenic protein. Its expression and regulation in leiomyoma is unknown.

Objective: To identify the expression pattern and regulation of EFEMP1 in LEIO versus MYO.

Design: Experimental - human tissue and primary cells.

Methods: Gene expression microarray was performed on matched sets of primary LEIO and MYO cells (n=3). One of the differentially expressed genes identified was EFEMP1. To validate EFEMP1's differential expression, RNA and protein were examined by real time PCR and western blots, respectively. Cells were treated with the demethylating agent, 5-Aza-2'-deoxycytidine (AZA), at varying concentrations (0-20uM) for five days ± the histone deacetylase inhibitor, trichostatin A (TSA), to determine if promoter hypermethylation and/or histone deacetylation play a role in the differential expression. RNA was isolated from the AZA/TSA treated cells and analyzed by real time PCR.

Results: 633 genes were differentially expressed ($p<0.01$) in the matched cell pair microarray. Validation of EFEMP1 revealed that mRNA was 3.19-fold lower in matched LEIO vs MYO (n=9; $p= 0.0001$) and 5.03-fold lower in LEIO in matched primary cells pairs (n=6; $p=0.0001$). Down regulation of EFEMP1 protein was confirmed by immunohistochemistry in matched tissue pairs, as well as western blots in matched cell protein. AZA treated leiomyoma cells caused up to a 4-fold increase in EFEMP1 gene expression in a dose response fashion (n=6, ANOVA $p=0.001$). TSA treatment revealed no additional increase in EFEMP1 expression.

Conclusion: The tumor suppressor and antiangiogenic gene EFEMP1 is expressed at significantly lower levels in LEIO versus MYO both in vivo and in vitro. Increases in gene expression in response to AZA treatment but not TSA, suggest that this down regulation is due in part to epigenetic regulation involving hypermethylation of the EFEMP1 promoter, but not to any alterations in histone deacetylase activity.

Support: This work was supported by the NIH WRHR Program, Northwestern Memorial Hospital, and the RWJ Foundation (EEM).

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HP1γ Phosphorylation by Aurora A Mediates Epigenetic Reprogramming of Cell Division in Cervical Cancer Cells. Phoebe H Leonard, Adrienne Grzenda, Ravi P Gada, Gaurang S Daftary, Raul Urrutia, Gwen A Lomberk. *Epigenetics in Reproductive Diseases Labs (ERDL), Department of Ob/Gyn, Mayo Clinic.*

Objective: We have reported that HP1γ phosphorylation at S83 via the cAMP-PKA pathway during interphase relocates this protein to euchromatin. Functionally, this mechanism is key for epigenetic reprogramming that occurs during senescence, which ultimately impacts on cell growth, aging, and cancer. HP1γ knockdown in cervical cancer cells results in mitotic aberrations and inhibits proliferation, whereas high HP1γ levels associate with enhanced proliferation. This study was designed to investigate the impact of Ser83 phosphorylation on cell division in cervical cancer cells.

Methods: HeLa cervical cancer cells were treated with aphidicolin or nocodazole to arrest cells in G1/S and G2/M, respectively. Double thymidine block was utilized to synchronize cells and analyze cell cycle progression by Western blot. In vitro Aurora kinase assays, Aurora A or B siRNA, and Aurora dominant negative constructs were used to analyze phosphorylation of Ser83-HP1γ. Localization of proteins was observed by immunofluorescence with specific antibodies. HP1γ, S83A or S83D-carrying adenovirus was used to infect cells prior to RNA extraction for microarray analysis.

Results: Phosphorylation of Ser83-HP1γ significantly increases upon entry into mitosis. During mitosis, P-Ser83-HP1γ colocalizes with Aurora A at the spindle poles and is phosphorylated by this kinase early in mitotic entry. Aurora B also phosphorylates this site in vitro but does not colocalize extensively with P-Ser83-HP1γ. P-Ser83-HP1γ also colocalizes with the cdk1/cyclin B complex, and HP1γ knockdown results in increased cyclin B1, cyclin B2, and cdk1 levels. Furthermore, we find that P-Ser83-HP1γ occupies the Cyclin B1 and B2 promoters, implicating this protein in direct transcriptional regulation of this complex. Overexpression of a non-phosphorylatable S83A or a phosphomimetic S83D HP1γ mutant triggers unique cell cycle-related gene expression networks, further highlighting the role that this phosphorylation has in cell division. The constitutively active S83D mutant also increases the rate of cell division, similar to wild type, whereas S83A abrogates this effect.

Conclusion: HP1γ phosphorylation at Ser83 plays a significant role in cell division and bears importance for understanding impairments which have been shown to be characterized by abnormally high levels of HP1γ, including cervical cancer.

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Treatment with Bone Marrow Derived Stem Cells (BMDSCs) Improves Fertility in a Murine Model of Asherman's Syndrome. Feryal A Alawadhi, Hugh S Taylor. *Department of Obstetrics and Gynecology, Reproductive Sciences, Yale University, New Haven, CT, USA.*

Objective: Asherman's Syndrome is characterized by intrauterine adhesions or fibrosis resulting as a consequence of trauma to the basal layer of endometrium. Mobilized of bone marrow-derived stem cells (BMDSCs) have been shown to contribute to the regeneration of endometrial tissue. Here we investigated the possibility that BMDSCs could regenerate endometrium and restore fertility in an animal model of Asherman's syndrome.

Methods: Asherman's syndrome was induced in female C57BL/6 mice by incising each uterine horn and curetting the endometium using a 27 gage needle. After 3 estrous cycles histological evidence of fibrosis was confirmed in the uterus of two mice. Another 20 C57BL/6 mice then underwent induction of experimental Asherman's syndrome and were subsequently randomized into two groups. BMDSCs were collected from long bones of 5 male LacZ transgenic and $1x10^7$ cells were injected to the tail vein of the BMDSC treatment group. The control group was injected with normal saline. After three estrous cycles the female mice were bred for a period of 3 months.

Results: In the treatment group 9 of the 10 mice conceived, while only 3 of 10 in the control group conceived (Chi-Square $p=0.0225$). The mean litter size was 6.3+/-1.4 in the treatment group and 5.3+/-4.0 in the control group and was not significantly different ($p=0.7$). There was also no significant difference in the time to conception between groups.

Conclusion: Asherman's syndrome is associated with infertility due to loss of normal endometrium. Stem cells derived from bone marrow are known to contribute to endometrium. He we demonstrate that BMSCs play a functional role in the uterus. Treatment with BMDSCs improves fertility after uterine injury. Transplantation with BMSC is a potential treatment for this disease.

O-137

Insulin Sensitivity and Adipose Tissue Metabolism in Morbidly Obese Pregnant Women. Sarah M Barr,[1] Shareen Forbes,[2] Nicholas M Morton,[2] Brian R Walker,[2] Jane E Norman.[1] *[1]Tommy's Centre for Maternal and Fetal Health, MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Midlothian, United Kingdom; [2]Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, Midlothian, United Kingdom.*

Maternal obesity affects approximately 30% of pregnancies in the UK and is associated with increased maternal and fetal morbidity and mortality, including gestational diabetes and pre-eclampsia. In non-pregnant obese subjects, insulin sensitivity is reduced, and is associated with elevated circulating leptin and pro-inflammatory cytokines. We hypothesize that in pregnancy, a state of progressive insulin resistance, obesity will lead to reduced insulin sensitivity and associated enhanced lipolysis in the third trimester.

We carried out hyperinsulinaemic euglycaemic clamp studies in healthy morbidly obese (BMI>40kg/m^2) pregnant women at 19 and 36 weeks (n=9), in lean (BMI <25kg/m^2) pregnant women at the same gestations (n=6), and in obese and lean non-pregnant women (n=7 per group). Whole body steady state glucose disposal was used as a surrogate measure of insulin sensitivity (IS). Anthropometric characteristics were recorded at each visit, including estimated fat mass using bioelectrical impedance. Fasting blood and subcutaneous adipose tissue were obtained at each visit and pro-inflammatory cytokines, adipokines and free fatty acids quantified.

At 19 weeks, lean pregnant women were significantly more insulin sensitive than morbidly obese pregnant women (8.4±1.4 vs 2.9±0.3 mg/kg/min, $p<0.001$). However, while there was an approximately 50% decrement in IS in lean women by 36 weeks, there was no such decrement in morbidly obese women; by 36 weeks there was no difference in insulin sensitivity between lean and morbidly obese pregnant women. Serum NEFA was significantly higher in obese compared with lean women at 19 weeks (0.53±0.03 vs 0.33±0.03mmol/L, $p<0.05$); by 36 weeks gestation, there was no significant difference between lean and obese pregnant women (0.50±0.05 vs 0.49±0.08 mmol/L).

We conclude that in morbidly obese women, pregnancy is not associated with a further decline in IS, so that by 36 weeks gestation IS is similar in lean and obese pregnant women. Therefore it is prolonged exposure to an insulin-resistant environment, with associated early pregnancy elevated circulating insulin and NEFA that contributes to the pathogenesis of obesity-associated complications rather than a more profound degree of late pregnancy insulin resistance.

O-138

Associations between Maternal Age and Obstetric and Perinatal Outcomes in Twin IVF Pregnancies. Eshanjit S Sapra,[1] Katherine J Hensel,[2] Inna V Landres,[1] Shirlee Jaffe,[1] Shari E Gelber.[1] *[1]Department of Obstetrics and Gynecology, Weill Cornell Medical College, New York, NY, USA; [2]Department of Pediatrics, Columbia University Medical Center, New York, NY, USA.*

OBJECTIVE: To investigate the effect of maternal age on obstetric and perinatal outcomes in twin IVF pregnancies.

STUDY DESIGN: We performed a retrospective cohort study of dichorionic twin IVF pregnancies in women ages 21-53 from January 2007 to June 2011 at an urban academic institution. Associations between maternal age at delivery and gestational age at delivery (GA), birth weight, IUGR, NICU admission, and preeclampsia (PEC) were evaluated. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using logistic regression.

RESULTS: Our study population included 468 dichorionic pairs. We compared the outcomes of twin IVF pregnancies in women <35 years (n=185, 39.5%), to women 35 to 39 years (n=161, 34.4%), 40 to 44 years (n=91, 19.4%) and 45 years and older (n=31, 6.62%). Women 45 years and older were at increased risk for PEC (OR: 3.228; 95% CI: 1.261, 8.263). However, after adjusting for use of donor eggs and nulliparity, age no longer increased PEC risk (AOR: 1.361; 95% CI: 0.417, 4.438). Use of donor eggs (AOR: 3.971; 95% CI:1.73, 9.118) and nulliparity (AOR: 3.147; 95% CI: 1.275, 7.771) were both independently associated with increased risk of PEC. There were no significant differences in birth weight, GA, NICU admission, or IUGR in either twin in women 40 to 44 years or 45 years and older compared to women younger than 35 years. Increased risks of birth weight <1500 g for one twin (OR: 2.933; 95% CI: 1.303, 6.606) and GA <33 weeks (OR: 2.14; 95% CI: 1.066, 4.293) were observed in women between 35 to 39 years compared to women <35 years. R-squared correlation between GA <33 weeks and birth weight <1500 g was 0.4128.

CONCLUSION: In twin IVF pregnancies, maternal age >40 is not an independent risk factor for poor perinatal outcomes including low birth weight, preterm delivery, IUGR or NICU admission. Risks of preterm birth and low birth weight are increased among women 35 to 39 years compared to women <35 years. After adjusting for use of donor eggs and nulliparity, maternal age >45 is not associated with PEC. Nulliparity and use of donor eggs are associated with increased risk of PEC among women >45.

O-139

A Polymorphism in the cias1 Gene Coding for an Inflammasome Component Influences Gestational Diabetes Mellitus (GDM)-Related Parameters in Pregnant Hispanic Women. Shirlee Jaffe,[1] Kanninen Tomi,[1] Jayaram Aswathi,[1] Diana Korneeva,[1] Normand Neil,[1] Rudge VC Marilza,[2] Steven S Witkin.[1] *[1]Obstetrics & Gynecology, Weill Cornell Medical College, New York, NY, USA; [2]Obstetrics & Gynecology, Sao Paulo State University, Unesp, Botucatu, Brazil.*

Introduction: The cias1 gene codes for the protein NALP3, the rate-limiting component in inflammasome formation. Inflammasome function has been postulated to influence the appearance of metabolic disease and diabetes. We postulated that a functional polymorphism in cias1 influences GDM-related parameters in pregnant Hispanic women.

Methods: Sera were obtained from 87 Hispanic pregnant women prior to undergoing the third trimester glucose challenge test (GCT) for determination of immune mediators by ELISA. DNA was obtained from buccal swabs for determination of a length polymorphism in cias1. Clinical and outcome parameters were obtained after completion of all testing.

Results: The allele frequency was 73.6% for allele 12, 14.4% for allele 7, 11.5% for allele 9 and 0.6% for allele 6. Possession of allele 9 was associated with an elevated mean one hour GCT result (127.3 vs. 109.4 mg/dL, p=0.0125) and a diagnosis of GDM (50.0% vs. 8.6%, p=0.0006). Women positive for allele 9 had a marginally significant elevated mean body mass index (BMI) (28.5 vs. 25.6, p=0.0577). There was no association between allele 9 and gestational age at delivery or mean neonatal birth weight. Women positive for allele 9 had lower serum levels of IL-1beta (<3.9 vs. 27.0 pg/ml, p=0.0333), 60kDa heat shock protein (0.8 vs. 3.8 pg/ml, p=0.0024) and visfatin (15.1 vs. 24.8 ng/ml, p=0.0303).

Discussion: The NALP3 inflammasome forms in response to infection or a non-infectious physiological stress. It induces production of IL-1beta, thereby activating a protective pro-inflammatory immune response. The association of cias1 allele 9 with reduced IL-1beta levels indicates that possession of this allele results in formation of a less efficient inflammasome. Women positive for cias1 allele 9 had increased detection of GDM and an elevated GCT and reduced levels of hsp60 and visfatin, known risk factors for GDM. Reduced inflammasome activity appears to increase susceptibility to GDM by elevating the occurrence of risk factors for this disorder. A decreased genetic capacity for IL-1beta production may interfere with fatty aid metabolism or increase persistence of a chronic infection that damages insulin-producing cells.

O-140

Metformin Does Not Prevent Diet-Induced Lipid Abnormalities in the Maternal Liver. Neeraj Desai,[1] Amanda Roman,[1] Burton Rochelson,[1] Madhu Gupta,[2] Malvika Solanki,[2] Xiangying Xue,[2] Christine Metz.[2] *[1]Maternal-Fetal Medicine, Hofstra North Shore-LIJ, NY; [2]Feinstein Institute for Medical Research, NY.*

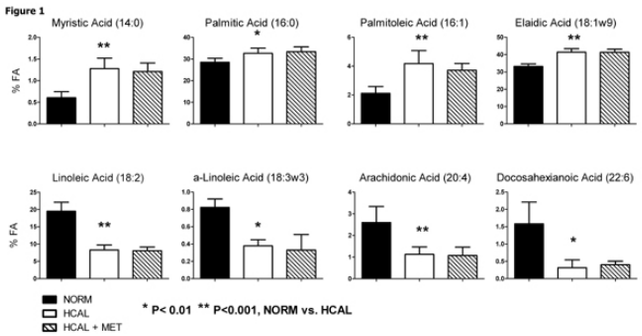
OBJECTIVE: Abnormal levels of fatty acids (FAs), particularly polyunsaturated fatty acids(PUFAs) are associated with increased risks of PTB, IUGR, and preeclampsia. Metformin(MET) can lower blood glucose and insulin, but also may improve lipid metabolism. Using an animal model of diet-induced obesity/metabolic syndrome during pregnancy, we investigated the effect of metformin on maternal lipid profiles.

METHODS: Female Wistar rats (6wk old) were fed normal(NORM) or high fat/high sugar(HCAL) diets for 5wks. After mating with NORM males, half of HCAL dams received MET(300mg/kg POdaily), and continued their respective diets throughout gestation(N=6-8/group). On GD19 dams were euthanized, maternal plasma and livers were analyzed for lipid profiles and total triglycerides(TG) and FAs, respectively. Liver sections were stained with Oil Red O to assess lipid deposition. Data were analyzed using Mann-Whitney.

RESULTS: HCAL dams gained more prepregnancy weight than NORM (61% vs.47%;p=0.03). HCAL dams had increased plasma TGs (597 vs. 379mg/dL, p=0.007), chol:HDL ratios (3.8 vs. 2, p<0.05), and decreased HDL (P<0.05). Unsaturated FAs and monounsaturated FAs were increased in the HCAL livers as compared to NORM; while PUFA were significantly decreased(fig 1). Liver TGs were significantly increased in HCAL vs. NORM (4.53 vs.3.22ug/mg, p<0.05). However, MET treatment had no impact on plasma lipids, liver FAs (fig 1) or total liver TGs(4.99 vs. 4.53ug/mg, p=0.64). Oil Red O staining confirmed increased lipid deposition in HCAL dams, irrespective of MET.

CONCLUSION: High fat and sugar diet leads to abnormal lipid deposition and FA ratios in the maternal liver in addition to obesity and altered plasma lipid profiles in the dam. Metformin treatment during pregnancy showed no effect. Maternal lipid changes may have a dramatic impact on the fetus because PUFAs, specifically DHA, are critical for normal fetal brain development and function. Future studies will investigate the effects of abnormal lipid metabolism on fetal/neonatal outcomes.

Figure 1



O-141

Omega-3 Supplementation in Obese Pregnant Women Reduces Placental mTOR Activation. Marilyn Galindo,[1] Francesca Gaccioli,[1] Susanne Lager,[1] Vanessa Ramirez,[1] Christiane Meireles,[1] Evelyn Miller,[1] Debra A Krummel,[2] Theresa L Powell.[1] *[1]Center for Pregnancy and Newborn Research, Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, TX; [2]Nutritional Sciences, University of Cincinnati, OH, USA.*

Introduction: Obesity affects approximately 30% of all pregnancies and is associated with fetal overgrowth, traumatic birth injuries and increased health risks in adulthood. We have recently reported that the placenta in obese mothers shows activation of the mTOR pathway, a cellular nutrient sensing

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pathway that regulates syncytiotrophoblast metabolism, protein synthesis and nutrient transport. Activation of placental mTOR in obese pregnancies could result in increased placental growth and nutrient transport leading to increased fetal growth. In the current study we hypothesized that supplementing obese mothers with the omega-3 fatty acid docosahexaenoic acid (DHA) reduces placental activation of mTOR. **Methods:** Women with pre-pregnancy BMI between 30 and 45 (n=25) were recruited in week 26 of pregnancy. Subjects were randomly assigned to placebo (corn/soy oil) or treatment (DHA, 800 mg/day) groups. The subjects were monitored at 26 weeks (baseline), 31 and 36 weeks of gestation. We collected placentas from 12 placebo and 10 DHA treated women who delivered at term. The activation state of the mTOR signaling pathway was studied in placental homogenates by evaluating phosphorylation of pathway intermediaries; 4E-BP 1 (Thr 37/46 and Thr 70), Ribosomal protein S6 (Ser235/236), and Akt (Ser 473, Thr 308) by western blot. Group differences were analyzed by t-test. **Results:** DHA supplementation from week 26 to term significantly increased maternal red blood cell phospholipid DHA content (5.1%) compared to placebo (2.5%, $p<0.001$). The phosphorylation of 4E-BP1 in the placenta was markedly reduced at both the Thr 70 ($-47 \pm 7\%$, $p<0.05$) and Thr 34/46 sites ($-34 \pm 6\%$, $p<0.05$) in the DHA supplemented group compared to placebo. There was no significant effect of DHA supplementation on the phosphorylation of Ribosomal protein S6, Akt Ser 473 or Akt Thr 308. **Conclusion:** Supplementation of obese pregnant women with the omega-3 fatty acid DHA reduced the activation of placental mTOR signaling. We speculate that reducing mTOR activation to the levels seen previously in lean women may contribute to normalization of placental function and could ameliorate the increased placental and fetal growth associated with maternal obesity.

O-142

Effect of Psycho-Education in Obese Pregnant Women on Pregnancy Outcomes, Randomized Controlled Trial. Annick Bogaerts,[1] Roland Devlieger,[2] Erik Nuyts,[3] Ingrid Witters,[4] Bea Van den Bergh.[5] *[1]Healthcare Research, KHLim-PHL University College, Hasselt, Belgium; [2]Obstetrics & Gynaecology, University Hospitals of KULeuven, Leuven, Belgium; [3]Healthcare Research, PHL University College, Hasselt, Belgium; [4]Prenatal Diagnosis, East Limburg Hospital, Genk, Belgium; [5]Psychology, Tilburg University, Tilburg, Netherlands.*

Background: In order to reduce perinatal complications in obese pregnant women, guidelines for adequate gestational weight gain (GWG) were developed.

Objective: to evaluate how a prenatal psycho-educational program for obese pregnant women affects GWG, method of delivery, birth weight as well as levels of anxiety and depression.

Methods: Randomization of 205 obese pregnant women into a control group, a brochure group and an experimental group, receiving 4 prenatal psycho-education sessions. Anxiety and depressed mood were measured during the first, second and third trimester of pregnancy. Multivariate linear regression (GWG), logistic regression (method of delivery), proportional odds models(birth weight) and linear mixed effects models (STAI/EDS), controlling for demographic variables and medical complications were used.

Results We found a significant reduction of GWG in the brochure and prenatal session group compared to the control group. Moreover, high motivation to change eating behavior, higher parity, stressful events in history and no alcohol consumption had a negative impact on GWG, while women with higher levels of depressed mood in the third trimester demonstrated higher GWG. For method of delivery and birth weight, no differences were demonstrated between three groups. Maternal age and state anxiety in the third trimester were negatively correlated with spontaneous delivery while a higher parity was positive correlated. For birth weight, non-spontaneous method of conception, hypertension and higher depressed mood in third trimester had significant lower odds of having a normal bith weight (2.5-4 kg). For evolution in levels of anxiety and depressed mood, no differences between the three groups of obese were mentioned. However, higher GWG and parity had significant positive effects on levels of anxiety and depressed mood, indicating interdependence of psychological variables and GWG.

Conclusion: These findings can justify the clinical implementation of a psycho-educational program in order to reduce GWG and psychological vulnerability in obese pregnant women.

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2-Methoxyestradiol Causes Functional Repression of Transforming Growth Factor β Signaling by Ameliorating Smad and Non-Smad Signaling Pathways in Uterine Fibroids Cells. Salama A Salama,[1] Concepcion R Diaz-Arrastia,[1] Gokhan S Kilic,[2] Marwa W Kamel,[2] Deepa A Patel.[1] *[1]Obstetrics &Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Background: Uterine fibroids (UF) are the most common benign tumors of premenopausal women. Transforming growth factor -beta (TGF-β) is a prominent etiological factor which is up-regulated in UF and mediates multiple events in the pathogenesis of this disease. TGF-β signaling is mediated by TGF-β receptor-specific phosphorylation of both the canonical, Smad-, and non-canonical, "non-Smad," pathways. Several studies have demonstrated that 2-methoxyestradiol (2ME2) exerts potent antifibrotic effects. However, the underlying mechanism of the antifibrotic effects of 2ME2 is unknown. In current study, we investigated whether 2ME2 can attenuate TGF-β effects on UF cells and to delineate the underlying molecular mechanisms.

Methods: Immunoblotting, real time RT-PCR, immunofluorescence, and immunoprecipitation, were used to investigate the effect of 2ME on TGF-β1 signaling in immortalized human uterine fibroid cells (huLM).

Results: Our data revealed that 2ME2 inhibits TGF-β-induced fibrogenic responses in immortalized human uterine fibroid cell (huLM). It reduces the basal and TGF-β1 (10ηg/ml)-induced expression of collagen type I(αI) [Col I(αI)], collagen type III(αI) [Col III(αI)], plasminogen activator inhibitor-1 (PAI-1), and connective tissue growth factor (CTGF). Similarly, 2ME2 reduces TGF-β-induced differentiation of huLM cells into myofibroblasts phenotype. Our data also show that 2ME2 exerts its antifibrotic effect by ameliorating both smad-dependent and samd-independent TGF- β1 signaling pathways. 2ME2 inhibits TGF-β-induced Smad2/3 phosphorylation and nuclear translocation. In addition, 2ME2 exerts profound inhibitory effects on non-smad effectors of TGF-beta Akt and mTOR.

Conclusion: 2ME2 inhibits TGF-β profibrotic effects and signaling by ameliorating both canonical and non-canonical signaling pathways that culminate in profibrotic effects of TGF-β. These findings suggest the potential usefulness of 2ME2 as anti-fibrotic therapy.

O-144

Estrogen Stimulates Hydrogen Sulfide Biosynthesis in Uterine Artery Endothelial Cells. Thomas J Lechuga,[1] Wen Wang,[1] Ronald R Magness, Dong-bao Chen. *[1]Depts of Ob/Gyn and Path, Univ CA, Irvine, CA, USA; [2]Dept of Ob/Gyn, Univ WI-Madison, Madison, WI, USA.*

Introduction: Estrogens potently dilate various vascular beds throughout the body, with the greatest responses occurring in the reproductive vasculature especially the uterine largely via stimulating endothelial vasodilator production. Endogenous hydrogen sulfide (H_2S), primarily generated from L-cysteine by the enzymes cystathionine g-lyase (CSE) and cystathionine b-synthase (CBS), has recently been implicated as a novel vasodilator in a variety of organs and tissues. However, it is unknown if estrogen regulates H_2S biosynthesis in the uterine artery endothelial cells (UAEC). **Objectives**: to determine if estrogen stimulates H_2S production by increasing the expression of its synthesizing enzymes in UAEC. **Methods**: Primary UAEC from late pregnant (d120-130) ewes were treated with increasing concentrations of estradiol-17b (E2b, 0-1 μM) for up to 72 hours. Total RNA and proteins were isolated to assess CSE and CBS steady-state mRNA and protein by quantitative real-time qPCR and Western blot analysis using specific antibodies, respectively. The methylene blue assay was used to determine H_2S production. **Results**: Treatment with E2b stimulated both CSE and CBS mRNA and protein expressions in a concentration and time-dependent fashion. Treatment with 1 to 100 nM E2b for 24 hours increased ($P<0.01$) both CSE and CBS mRNA and protein expression. Significant increases of CSE and CBS mRNA and protein occurred at 24 hours with 10 nM E2b treatment and continued to increase up to 72 hours. In concert with E2b- induced rises in the expression of CSE and CBS, E2b treatment also stimulated H_2S production ($P<0.05$). Pretreatment with ICI 182, 780 blocked estrogen stimulation of both CSE and CBS mRNA and protein expression and H_2S production. **Conclusion:** Estrogen stimulates H_2S production by upregulating CSE and CBS expression via specific receptor-dependent mechanism in UAEC. These data suggest that H_2S may function as a novel endothelium-derived factor for mediating estrogen stimulation of uterine vasodilatation during gestation (Supported by HL70562 & HL98746).

O-145

Blockade of TGF-β Prevents Repression of BMP-2 Mediated HOXA10 Expression by Fibroid Conditioned Media in Endometrial Cells. Leo F Doherty, Hugh S Taylor. *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: Endometrial receptivity is impaired in women with fibroids. We have previously shown that TGF-β3, secreted by fibroids, decreases endometrial receptivity by altering the endometrial response to BMP-2. BMP-2 regulates HOXA10, a transcription factor necessary for embryo implantation. Here we examined the effect of TGF-β blockade on expression of HOXA10 after exposure to fibroid-conditioned media (F-CM) in BMP-2 treated endometrial stromal cells (ESC).

Methods: Human ESC and fibroid cells were isolated from surgical specimens. Two approaches were utilized to block TGF-β. In the antibody-mediated approach, ESC were treated with F-CM that was pre-incubated with TGF-β pan-specific antibody to block TGF-β3 (treatment), F-CM pre-incubated with non-specific rabbit IgG (negative control), or non-fibroid conditioned media. In the transfection-mediated approach, ESC were transfected with a TGF-β receptor type II mutant (K227R mutation) expression vector or empty vector (control). Following transfection, cells were treated with F-CM or standard media. After 24 hours of F-CM treatment, all cells were treated with recombinant human BMP-2. qRT-PCR was used to determine HOXA10 expression. PCR data was compared using Student's t-test.

Results: HOXA10 expression was significantly decreased in cells following treatment with F-CM (0.63 fold, $p<0.05$). Pre-incubation with rabbit IgG did not prevent HOXA10 repression. HOXA10 repression was prevented by pre-incubation of F-CM with the neutralizing TGF-β antibody (0.93 fold, $P>0.05$). Similarly, F-CM treatment significantly decreased HOXA10 expression in cells transfected with empty plasmid vector, compared to cells not exposed to F-CM (0.33 fold, $p<0.05$). HOXA10 expression increased after F-CM treatment in cells transfected with mutant TGF-β receptor II (1.65 fold, $P<0.05$).

Conclusion: TGF-β3, secreted by fibroids, impairs endometrial receptivity by altering BMP-2 signaling. HOXA10 expression was unresponsive to BMP-2 treatment in cells exposed to F-CM. Blockade of TGF-β3 by neutralizing antibody or transfection with a non-functional TGF-β receptor prior to exposure to F-CM prevented repression of BMP-2 mediated HOXA10 expression. TGF-β3 is necessary to mediate the adverse effects of fibroids on endometrial cells. TGF-β3 blockade in the endometrium of women with fibroids may present a novel treatment strategy to improve endometrial receptivity.

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MicroRNA-29 Expression and Function in the Pathogenesis of Leiomyoma. Erica E Marsh, J Brandon Parker, Ju Wu, Artis Lewis, Serdar E Bulun. *Obstetrics and Gynecology, Feinberg School of Medicine - Northwestern University, Chicago, IL, USA.*

Background: Leiomyoma are benign uterine tumors characterized by excessive extracellular matrix (ECM), however, the mechanisms behind this are poorly understood. MicroRNAs (miRs) are small noncoding RNAs that repress gene expression. Several miRs have been shown to be differentially expressed in leiomyoma (LEIO) versus myometrium (MYO), but few studies have linked phenotype to this differential expression. MiR-29 is known to regulate collagen expression in other fibrotic diseases but its role in leiomyoma is currently unknown.

Objective: To determine the expression and impact of miR-29 on ECM in LEIO versus MYO.

Design: Experimental - human tissue and primary cells

Methods: LEIO and MYO tissue was obtained and either flash frozen or digested for the isolation of cells. Real time PCR was performed to determine the relative expression of miR-29 in LEIO versus MYO in vivo. LEIO cells were transfected with miR 29 precursors to overexpress miR-29. MYO cells were transfected with miR-29 silencing RNAs to down regulate miR-29 expression. PCR and westerns were performed on the transfected cells to determine whether miR-29 regulates collagen production.

Results: Real time PCR confirmed that miR-29 was significantly down regulated in LEIO versus MYO in vivo ($n=8$; $p< 0.001$). PCR also confirmed successful overexpression of miR-29 in LEIO cells (>1000 fold; $p< 0.0001$) versus control and successful down regulation of the miR-29 species in MYO cells with the transfection of miR precursors and silencers respectively. Real time PCR of transfected LEIO cells revealed no differences in COL1A1, COL2A1 and COL3A1 (major collagens) gene expression ($p>0.05$; $n=3$). However, western blots of transfected LEIO cells demonstrated selective down regulation of the major collagens with overexpression of miR-29.

Conclusions: Mir-29 is down regulated in LEIO versus MYO. Restoration of miR-29 expression in LEIO can be achieved and results in a decreased major collagen expression at the protein level but not at the mRNA level suggesting inhibition of translation but not degradation of the major collagen RNAs by miR-29. Dysregulation of miR-29 in LEIO is a novel and potentially important mechanism contributing the pathologic ECM production in these prevalent tumors.

Support: This work was supported by the NIH WRHR Scholars Program, Northwestern University, Northwestern Memorial Hospital and the RWJ Foundation (EEM).

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MiR-200c Down-Regulated Leiomyoma IL-8 Expression and Cell Proliferation through Ikk β-NFκB Signaling Pathway and Cyclin D1/E Repression. Tsai-Der Chuang, Nasser Chegini. *OB-GYN, UF, Gainesville, FL, USA.*

Enhanced inflammation and cell proliferation are central to development and progression of tumorigenesis and are closely associated with aberrant expression of miRNAs. The objective of this study was to assess the regulatory function of miR-200c in the above processes in leiomyomas (LYO) as compared to myometrium (MYO). Using LYO and paired MYO (N=79) from un-treated (proliferative, secretory phase and irregular uterine bleeding) and women who received different hormonal therapies [GnRHa, Depo-Provera, oral contraceptives (OCPs) and progesterone] we found a lower expression of miR-200c in 69.8% (30/43) of LYO from untreated group($p=0.0462$) and 55.6% (10/18), 88.9% (8/9), 50% (1/2) and 28.6% (2/7) of LYO from Depo-Provera, GnRHa, progesterone and OCPs treated group, respectively. miR-200c expression was also lower in 70.8% (17/24) of LYO from Caucasian ($p=0.0431$) as compared to 62.6% (10/16) of tissues from African Americans ($p=0.1735$). In contrast, IL-8 expression was upregulated in 63.4% (26/41) of LYO from untreated group and 33.3% (4/12), 16.7% (1/6), 0% (0/2), 0% (0/3) from Depo-Provera, GnRHa, progesterone and OCPs treated groups, respectively without any ethnic differences [66.7% (12/18) vs 60% (12/20)] between Caucasians and African Americans. Using real-time PCR, western blotting, luciferase reporter assay, ELISA, CHIP assay, immunoprecipitation, cellular sub-fractionation and proliferation assay, we identified that gain-of function of miR-200c in isolated MYO and LYO smooth muscle cells (MSMC and LSMC) (N=15) regressed IL-8 expression and secretion without interacting with IL-8 3'UTR. However, miR-200c induced IL-8 repression through inhibition of IKKβ expression at protein but not mRNA level by directly interacting with IKKβ 3'UTR and decreased NFκB activity leading to lower NFκB binding to IL-8 promoter. miR-200c also induced E-cadherin trapping NFκB at cellular phase resulting in decreased NFκB nuclear translocation. Gain-of function of miR-200c also resulted in decreased rate of cell proliferation through downregulation of cyclin D1 and cyclin E. In conclusion, we demonstrated that miR-200c and IL-8 expression are inversely correlated which to some extend was hormonally- and ethnically-dependent, and provide evidence for a novel molecular mechanism involving IKKβ expression and NFκB activity by which miR-200c functionally regulates leiomyomas cellular proliferation and IL-8 expression. Supported by NIH: HD37432 and HD58664

O-148

Novel MED12 Gene Mutations in Southern USA Women with Symptomatic Uterine Fibroids. Sunil K Halder, Chakradhari Sharan, Waseem Khoder, Ayman Al-Hendy. *Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*

Background: Uterine leiomyomas (fibroids) are the most common benign tumors that affect millions of women globally and these tumors can cause significant symptoms including excessive vaginal bleeding, preterm birth and recurrent abortion. Uterine leiomyomas occur at the rate of three to four times higher incidence in black women than their counterpart white women. However, the major causes of this complicated disease are still unknown. The mediator complex (Med12) is a 26-subunit transcriptional regulator that bridges DNA regulatory sequences to the RNA polymerase II initiation complex. Although a recent study (Science 2011, DOI: 10.1126/science 1208930) demonstrated that the Med12 gene mutations are associated with fibroid tumorigenesis in Finland, no information is available about Med12 mutation in Southern American patients with symptomatic uterine fibroids.

Objective: To study the genetic basis of uterine leiomyoma tumor development in American women.

Design: To identify Med12 gene mutation we isolated genomic DNAs from 47 uterine fibroid tumors and 44 normal myometrium tissue samples from 47

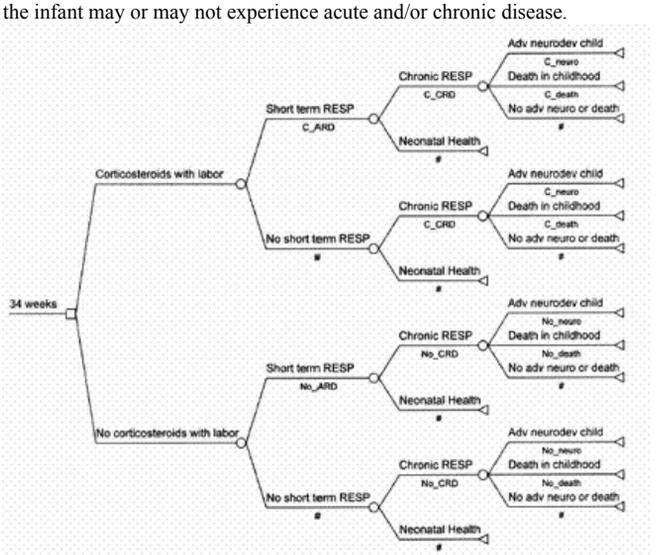
women undergoing hysterectomy from Southern USA. These DNA samples were amplified by polymerase chain reaction (PCR) using Med12 exon2 specific primers. PCR products were purified and then analyzed for Med12 gene mutation.
Results: By sequence analyses of uterine fibroids, we determined that the Med12 gene is mutated in 74.4% of all fibroid tumors (including deletion mutation). These mutations include 107 T>G (4.3%), 130 G>C (2.1%), 130 G>A (6.4%), 131 G>C (2.1%), 131 G>A (23.4%), and 131 G>T (2.1%). Interestingly, we found four unique mutations in these patients and these mutations are 107 T>C (12.8%), 105 A>T (2.1%), 122 T>A (2.1%), and 92 T>A (2.1%). Additionally, we found higher rate of deletion mutation (14.9%) in the above fibroid patients. All these mutations were found in exon2 region of the Med12 gene and these mutations can cause Med12 gene malfunction. Analysis of possible correlation of specific mutations with sever tumor burden as well as certain ethnicity is currently ongoing in our laboratory and will be presented at the meeting.
Conclusion: The aberrant function of Med12 gene may be involved in fibroid tumorigenesis in Southern USA women with uterine fibroids.
Support: RCMI pilot 2G12RR003032-26, MeTRC/CRC pilot 202142-535001-20, and NIH/NICHD R01 HD046228.

O-149

The Association of Sex Steroid Hormones and Levels of Leptin and Leptin Receptor in Ethnic Minority Postmenopausal Women. Kathleen Brennan,[1,2] Brian Chen,[1] Lauren Nathan,[2] Anthony Butch,[3] Simin Liu.[1,2,4] *[1]Epidemiology, UCLA, LA, CA, USA; [2]OBGYN, UCLA, LA, CA, USA; [3]Pathology and Laboratory Medicine, UCLA, LA, CA, USA; [4]Medicine, UCLA, LA, CA, USA.*
PURPOSE: Menopause is marked by changes in sex hormone levels, as well as weight gain and increased body fat. Leptin plays an important role in the regulation of body weight homeostasis. However, the interrelationship of sex hormone levels and levels of leptin and leptin receptor is largely unknown, especially in minority women.
METHODS: Among 82,069 postmenopausal women aged 50 to 79 years enrolled in the Women's Health Initiative Observational Study, a cross-sectional analysis of the relationships between baseline sex steroid hormone levels and levels of leptin and soluble leptin receptor, and the modulation of these associations by hormone replacement therapy (HRT), were assessed in a nested case control sample of 1791 apparently healthy ethnic minority women. During a median follow-up of 5.9 years, 589 women who developed clinical diabetes were matched to 1202 study participants who were free of disease. All hormone levels were log transformed to enhance regression assumptions.
RESULTS: Estradiol (E2) levels (log pg/ml) were directly associated with leptin levels (log ng/ml) (β=0.07, p<0.0001), even when controlling separately for BMI, interleukin-6, tumor necrosis factor-α-receptor 2, or insulin. However, controlling for C-reactive protein (CRP) attenuated this association. Total testosterone (TT) levels (log ng/ml) were also directly associated with leptin levels (β=.05, p=.004), except when controlling for BMI. When stratifying by HRT status, E2 levels were directly associated with leptin levels in all women except current users of HRT(β=0.14-.16, p=0.0001). TT levels were inversely associated with leptin receptor levels in current users of HRT (β=-0.04, p=.004), but not in past or never users; the association was weakened when controlling for BMI. Sex hormone binding globulin (SHBG) had a strong inverse association with leptin levels (β=-0.27, p<0.0001) but was directly associated with leptin receptor levels (β=0.12, p<0.0001).
CONCLUSIONS: E2 levels were directly associated with leptin levels except when controlling for CRP or in current users of HRT. The relationships between TT, leptin, and leptin receptor may be mediated by BMI. Higher SHBG levels were associated with lower leptin levels and higher leptin receptor levels.

O-150

Is the Administration of Corticosteroids Cost Effective for Late Preterm Infants? Jamie A Bastek,[1] Holly Langmuir,[1] Laxmi A Kondapalli,[1] Emmanuelle Pare,[2] Joanna E Adamczak,[1] Sindhu K Srinivas.[1] *[1]Maternal & Child Health Research Program, OBGYN, CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Division of MFM, Department of OBGYN, University of British Columbia, Vancouver, BC, Canada.*
Introduction: ACOG recommends the administration of a single 48-hour course of antenatal corticosteroids (ACS) to all patients 24-33 6/7 weeks at risk for preterm birth. Although infants born during the late preterm period are also at increased risk of adverse respiratory outcomes compared to term infants, current data does not support giving ACS between 34-36 weeks. Our objective was to perform a decision analysis to determine whether the administration of ACS in labor were cost effective between 34-36 weeks.
Methods: Separate decision trees were created to represent each week in consideration - example below. The choice node involved whether or not to administer ACS with preterm labor (PTL). In either strategy, we assumed that the infant may or may not experience acute and/or chronic disease.



We conducted a literature review to estimate the probabilities of clinical outcomes and the costs and utilities associated with different health states. Base-case cost-effectiveness analysis was performed to compare each strategy at each gestational age. Given that without tocolysis most deliveries will occur within 48 hours, analyses were repeated with adjusted probabilities that assumed exposure to only a partial course of ACS. A threshold of $100,000/QALY was considered cost-effective.
Results: The incremental cost-effectiveness ratio (ICER) favored the administration of ACS at 34, 35, and 36 weeks (ICER $65,156/QALY, $66,880/QALY, and $67,054/QALY respectively). If only a partial course were received, it was favorable to administer ACS at 34 weeks (ICER $51,254.67/QALY) but not at 35 or 36 weeks ($139,065/QALY, $139,256/QALY respectively).
Discussion: While a full course of ACS is a cost effective strategy for patients 34-36 weeks, even a partial course appears cost-effective at 34 weeks. The results from the ongoing MFMU RCT to address the risk/benefit profile of ACS in the late preterm period are urgently needed.

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Previous Pregnancy Loss Is a Significant Risk Factor for Adverse Pregnancy Outcomes: Evidence from a Large Prospective Cohort. Fergus P McCarthy,[1] Ali S Khashan,[1] Robyn A North,[2] Philip N Baker,[3] Gus Dekker,[4] Lucilla Poston,[2] Louise C Kenny,[1] Keelin O'Donoghue.[1] *[1]The Anu Research Centre, University College Cork, Cork, Ireland; [2]Women's Health Academic Centre, King's College, London, United Kingdom; [3]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada; [4]Obstetrics and Gynecology, Adelaide University, Adelaide, Australia.*
Objectives
Women with recurrent pregnancy loss (>three miscarriages) are known to be at increased risk of preterm birth and fetal growth restriction. This study aimed to clarify the association between women with either one or two and three previous miscarriages and subsequent adverse pregnancy outcomes.
Methods
This prospective cohort study consisted of 3531 nulliparous women recruited in the multicentre Screening for Pregnancy Endpoints (SCOPE) study.[1] Women with 1 and 2 or 3 previous miscarriages were compared with women who had no previous miscarriages. Outcomes included spontaneous preterm birth (SpPTB), pre-eclampsia (PE), small for gestational age (SGA), large for gestational age (LGA) and placental abruption. Logistic regression was used for data analysis.
Results
In the study cohort, 2624 women had no previous miscarriage (reference group), 691 had 1 previous miscarriage and 216 had 2 or 3 miscarriages. The estimates of the associations between previous miscarriage and the outcome measures are summarized in Table 1.

Table 1: The association between previous miscarriage and adverse pregnancy outcomes

	1 miscarriage (n=691) Adjusted OR (95% CI)	2 or 3 miscarriages (n=216) Adjusted OR (95% CI)
Pregnancy Outcomes		
Pre-eclampsia	1.0(0.7,1.5)	1.1(0.6,1.9)
Spontaneous preterm birth	1.2(0.8,1.8)	2.4(1.5,3.8)
Small for gestational age	1.4(1.1,1.8)	1.1(0.7,1.8)
Large for gestational age	1.1(0.8,1.4)	0.9(0.5,1.5)
Placental abruption	1.3(0.5,3.1)	6.1(2.8,13.4)

All figures adjusted for maternal age, smoking, alcohol, ethnic origin, BMI and SCOPE centre

Conclusion

Women with previous miscarriages in pregnancy are at increased risk of adverse pregnancy outcomes including an increased incidence of SpPTB, SGA and placental abruption compared to women with no previous miscarriages. Final analysis will include data from the final SCOPE cohort of 5690 women.

References

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North et al. BMJ. 2011;342:d1875. doi: 10.1136/bmj.d1875.PMID:21474517

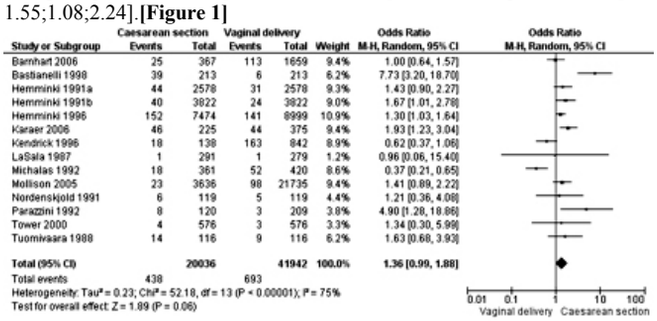
O-152

Cesarean Section & Subsequent Ectopic Pregnancy-Systematic Review & Meta-Analysis. Sinead M O'Neill,[1] Louise C Kenny,[2] Ali S Khashan,[2] Richard A Greene,[1] Tine B Henriksen,[3] Jennifer E Lutomski,[1] Patricia M Kearney.[4] *[1]NPEC, University College Cork; [2]Anu Research Center, University College Cork; [3]Perinatal Epidemiology Research Unit, Department of Pediatrics, Aarhus University Hospital, Denmark; [4]Department of Epidemiology&Public Health, University College Cork.*

Background: Ectopic pregnancy,the leading cause of pregnancy-related death in the first trimester occurs in 1-2% of reported pregnancies[1].Cesarean section may be a potential risk factor.The aim was to examine the relation between Cesarean section and subsequent ectopic pregnancy using a systematic review and meta-analysis.

Methods:PubMed, Embase, CINAHL, Web of Knowledge,Cochrane Library, Medline and Scopus were searched from their inceptions,as well as reference lists of identified studies.Eligibility criteria were a comparison of vaginal delivery and Cesarean section and reporting of ectopic pregnancy.Two assessors independently reviewed titles,abstracts & full articles to identify any eligible papers,with differences resolved by consensus.Data were extracted using a standardized data collection form.Revman software was used to combine odds ratios (ORs),generating an overall effect size,as well as *a priori* defined sensitivity analyses.

Results:13 studies conducted between 1973 & 2005 were included (n=61,978). An increased risk of ectopic pregnancy following Cesarean section was found in the fixed effect model [OR 1.28;95% Confidence interval (CI) 1.12;1.46]. The random effects model showed an increased risk but not significantly [OR 1.28;0.99;1.88].Risk of ectopic pregnancy was significantly increased in the following sensitivity analyses;8 cohort studies [OR 1.38;1.16;1.64]; 6 studies with primiparous women [OR 1.37;1.15;1.64]; and 11 European studies [OR 1.55;1.08;2.24].**[Figure 1]**



Conclusions:In this systematic review and meta analysis, women having a Cesarean section had increased risk of ectopic pregnancy compared to women who had vaginal delivery. However, further research is warranted as these studies are limited by small sample sizes and the fact that many were conducted some time ago.

References:

1. Lozeau&Potter. Am Fam Physician, 2005.72(9):p.1707-8.

O-153

The Correlation between Neonatal Body Fat Percentage and Neonatal Biometric Measures. Colum R Keohane,[1] Ali S Khashan,[1] Fergus P McCarthy,[1] David Broadhurst,[2] Mairead Kiely,[3] Deirdere M Murray,[4] Jonathan O'B Hourihane,[4] Louise C Kenny.[1] *[1]ANU Research Centre, University College Cork, Cork, Ireland; [2]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada; [3]School of Nutritional and Food Sciences, University College Cork, Cork, Ireland; [4]Paediatrics and Child Health, University College Cork, Cork, Ireland.*

Background: Variation in fetal size can have far reaching implications for both mother and child. Both large and small for dates fetuses carry increased risks of adverse obstetric outcomes. Many measures are used to quantify fetal size and growth. Air displacement plethysmography has been validated as an accurate, reproducible, non-invasive method of measuring body fat percentage. The role of body fat percentage remains unclear. The aim of the study was to determine the correlation between neonatal body fat percentage (%BF) with each of birthweight (Bwt), infant body mass index (BMI), individualised birthweight ratio(IBR), ponderal index (PI) and neonatal head circumference.
Methods: Study participants were drawn from the BASELINE (Babies After Scope: Evaluating Longitudinal Indices Using Neurological and Nutritional Endpoints) study in Cork (www.baselinestudy.net). %BF was measured using the Peapod air displacement plethysmograph within the first four days of life. Pearson Correlation coefficients were calculated, after linearity and normal distribution were confirmed, for each of %BF, Bwt, BMI and head circumference. Spearman correlation coefficient was calculated for IBR.
Results: 1240 infants were born to first time mothers enrolled in BASELINE during the study period (609 female and 634 male). The principle measures were all found to be normally distributed. Pearson's Correlation coefficients showed a significant correlation between %BF and Bwt, IBR, BMI, PI and head circumference (Table).

Correlation between %BF and neonatal biometric measures

	%BF	Bwt	IBR	BMI	PI	Head Circumference
Mean (SD)	11.1% (4.1)	3507 grams (468)	50.1(1.2)	13.8(1.2)	2.7(0.2)	34.8 cm(1.4)
Correlation coeff with %BF	1	0.48	0.45	0.44	0.33	0.25
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Conclusion:This study demonstrates a significant correlation between %BF and other neonatal bimetric measurements. Ongoing work is exploring the correlation between %BF and adverse maternal and neonatal outcomes and infant and childhood ill health.

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Influence of Fetal Sex on Maternal Anti-Mullerian Hormone Levels. Donna Santillan,[1] Ryan Empey,[1] Mark K Santillan,[1] Eric Tyler,[2] Stephen Hunter,[1] Elaine M Smith,[3] Barbara J Stegmann.[1] *[1]OB/GYN, University of Iowa Hospitals and Clinics, Iowa City, IA, USA; [2]Carver College of Medicine, University of Iowa, Iowa City, IA, USA; [3]Epidemiology, University of Iowa, Iowa City, IA, USA.*

Introduction: Maternal anti-mullerian hormone declines sharply between 13-15 weeks, likely as a result of feto-placental signaling. Fetal AMH levels are known to be widely disparate after the first trimester, with high levels in male and absent levels in female. However, it is unclear as to whether differing fetal AMH levels influence the pattern of change of maternal AMH. Our objective was to examine AMH throughout gestation to determine if the maternal concentration varies according to the gender of the fetus. **Methods:** De-identified maternal plasma samples along with demographic and pregnancy outcome data were obtained from the IRB-approved Maternal-Fetal Tissue Bank at the University of Iowa. All women were ≥18 years old and had an uncomplicated singleton delivery at ≥37weeks. AMH was tested using the GenII AMH ELISA assay (Beckman Coulter). Bicinchoninic acid (BCA) assay (Pierce) was used to measure total protein. AMH was normalized to total protein prior to analysis. Mean AMH and AMH by gestational age between women carrying boys vs. girls was compared with logistic regression modeling.
Results: 154 samples from 107 women (51 boys and 56 girls) were analyzed. Because of multiple sampling, 78 samples were from boys and 76 samples were from girls. No differences in maternal age, gestational age at delivery, or number of samples from each gestational category between sexes. Mean AMH levels were not different if carrying a male vs female fetus (p=0.12). However, when stratified by gestational age, mean AMH (±SEM) at 11-15 weeks was significantly higher in women with male fetuses (2.0±0.30ng/mL) vs. female fetuses (0.94±0.20ng/mL) (p=0.008).

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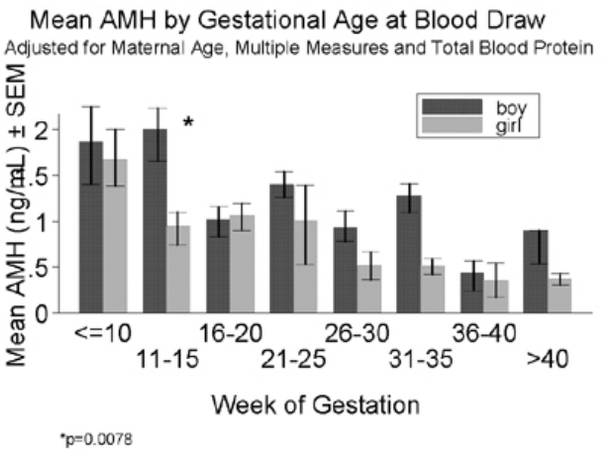

Conclusion: AMH falls in pregnancy between 11-15 weeks regardless of fetal sex; however, maternal AMH is significantly higher in pregnancies carrying male compared to female fetuses. This may represent a sexually dimorphic response in the ovary to feto-placental signaling.

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Maternal Protein Restriction Results in Altered Transcriptional and Epigenetic Regulation of Hepatic Liver X Receptor (LXRα) Target Genes Leading to Impaired Glucose Homeostasis in Adult Rat Offspring. (Peter) Thin X Vo, Gurjeev Sohi, Andrew Revesz, Daniel B Hardy. *Children's Health Research Institute, Depts of Ob/Gyn & Physiology/Pharmacology, The University of Western Ontario, London, ON, Canada.*

Epidemiological studies have correlated intrauterine growth restriction (IUGR) and the prevalence of chronic diseases such as the Metabolic Syndrome. However, the molecular mechanisms which underlie how IUGR leads to the long-term development of these diseases remain elusive. Previously we and others have demonstrated that a maternal protein restricted (MPR) diet (8% protein) during both pregnancy and lactation leads to decreased birth weight, high cholesterol and impaired glucose homeostasis in the offspring. It has recently been discovered that the Liver X Receptor (LXRα) regulates glucose homeostasis by impairing critical genes involved in gluconeogenesis (glucose-6-phosphatase [G6Pase], 11β-hydroxysteroid dehydrogenase type 1 [11β-HSD1], and phosphoenolpyruvate carboxykinase [PEPCK]). Therefore, we hypothesized that the expression of LXRα-target genes involved in glucose homeostasis would be altered by MPR, leading to augmented circulating glucose in later life. Dams in the control group were given a diet with 20% protein throughout pregnancy and weaning, while mothers in the MPR group were given a low protein diet during gestation. LP2 offspring received a control diet post-weaning, while LP3 offspring received a control diet immediately after birth. Glucose tolerance tests revealed impaired glucose tolerance for both MPR offspring (LP2 and LP3) at postnatal day 130. Western blotting revealed that LXRα expression was decreased ($p<0.05$) at postnatal day 130 in MPR male offspring while expression of 11β-HSD1 and G6Pase were significantly higher ($p<0.05$) in LP3 males. Chromatin immunoprecipitation revealed that binding of LXRα to the putative LXRE on the *G6Pase* promoter was decreased ($p<0.05$) in LP3 males, along with increased acetylation of Histone H3 [K9,14], a hallmark of chromatin opening. In summary, our study found enhanced expression of critical genes involved in hepatic gluconeogenesis in MPR offspring, concomitant with altered transcriptional and epigenetic regulation of these genes, and ultimately, impaired glucose tolerance. Moreover, LP3 animals had the worst glucose tolerance, suggesting that early restoration in protein during lactation exacerbates these fetal programming effects on G6Pase and 11β-HSD1. Supported by CIHR.

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Paradoxical Effects of NPY on Hypothalamic Arcuate (ARC) Neurons Mediated by Potentiated NPY Receptors in Growth Restricted (IUGR) Rats. Xiaoping Sun, Tatsuya Fukami, Tie Li, Mina Desai, Michael G Ross. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Neural plasticity in developing offspring allows both structural and functional adaptive changes to environmental stimuli. IUGR offspring have a programmed propensity for hyperphagia and adult obesity. Neuropeptide Y (NPY) action within the hypothalamic arcuate nucleus (ARC) regulates orexigenic responses. We sought to determine the effects of NPY on ARC neurons in IUGR and control weanling rats.

Study design: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. Hypothalamic brain slices from 6 control and 6 IUGR pups (3 male and 3 female for each group) at 21-27 days were used to characterize the effects of NPY on ARC neurons by whole-cell patch clamping techniques. Membrane potential and spontaneous excitatory and inhibitory postsynaptic membrane potentials (EPSC/IPSC) were recorded before and after NPY application (0.5-1μM).

Results: NPY had differential impact on ARC neurons in control and IUGR rats. NPY hyperpolarized ARC neurons to a greater degree in IUGR rats than that in controls (9.1 ± 2.0; $n=14$ vs 3.0 ± 1.0 mV; $n=12$, $P<0.02$). Spontaneous EPSCs recorded from NPY neurons had a paradoxically lower frequency in IUGR rats than in controls (6.3 ± 0.9 vs. 10.4 ± 2.0 Hz, $P<0.05$), though there was no difference in spontaneous IPSCs.

Conclusion: Our results show that the NPY receptor responses are enhanced in IUGR rats, potentially contributing to increased appetite. We propose that NPY release may play dual roles in regulation of ARC neurons via presynaptic Y2 and postsynaptic Y1 receptors, leading to decrease of presynaptic glutamatergic input and lower membrane potential.

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Maternal Glucocorticoid Treatment Modifies the Methylome of Offspring and Alters Expression of Key Methylation-Related Enzymes in a Tissue-Specific Manner and across Multiple Generations. A Crudo,[1,2] A Kostaki,[3] M Szyf,[1,2] SG Matthews.[3,4,5] *[1]Pharmacology & Therapeutics, McGill University, Canada; [2]Sackler Program for Epigenetics & Psychobiology, McGill University; [3]Physiology, ; [4]Obstetrics & Gynecology, ; [5]Medicine, University of Toronto, Canada.*

Objective: Synthetic glucocorticoids (sGC) are given to pregnant women at risk of delivering pre-term. Animal studies revealed that sGC exposure can cause life-long changes in endocrine function and behavior, which involve long-term changes in gene expression. We hypothesized that; 1) antenatal sGC treatment alters DNA methylation in the first generation (F1) juvenile and adult offspring, and this is maintained in the second generation (F2); 2) sGC treatment permanently programs genes involved in epigenetic modulation and this manifests across generations.

Methods: Pregnant guinea pigs were s.c injected with Betamethasone (1 mg/kg) or saline vehicle on GD 40, 41, 50, 51, 60 & 61. Animals delivered undisturbed. One group of male offspring was euthanized at 10 days of age (PND10) and another at 90 days of age (n=4 per group). Adult F1 females were mated with naïve males. F2 adult males were euthanized at 90 days of age (n=3). Global methylation was analyzed with the Luminometric Methylation Assay (LUMA). mRNA expression of genes associated with epigenetic regulation was determined using qRT-PCR.

Results: Antenatal sGC treatment significantly decreased global methylation in the PND10 liver, adrenal and cerebellum, and increased global methylation in the kidney ($P<0.05$). Adult animals prenatally exposed to sGC had significant global hypomethylation in all tissues ($P<0.05$). Moreover, global hypomethylation was observed in Beta exposed F2 animals ($P<0.05$). Further, mRNA expression studies in the kidney and cerebellum revealed a significant effect of antenatal sGC therapy on the expression of genes involved in epigenetic regulation ($P<0.05$). MBD2 and TET1, two genes associated with demethylation, showed substantial increases in mRNA expression.

Conclusion: This is the first study to show that fetal sGC exposure can lead to permanent changes in the DNA methylation in the liver, kidney, adrenal and cerebellum. It is particularly striking that similar methylation changes were present in F2 offspring. Further, it appears that sGC exposure modifies the epigenetic machinery by modifying the expression of genes that are key to epigenetic regulation.

Supported by:The Canadian Institutes of Health Research

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Complement Component C5a Mediates Adverse Cognitive Outcomes in Offspring Following In Utero Exposure to Experimental Placental Malaria. Chloe R McDonald,[1,2] Karlee L Silver,[2] Keith T Ho,[3] Howard T Mount,[3] Kain C Kain.[1,2] *[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada; [2]McLaughlin-Rotman Centre for Global Health, University Health Network, Toronto, ON, Canada; [3]Physiology, University of Toronto, Toronto, ON, Canada.*

Each year approximately 125 million pregnancies are at risk of complications due to malaria infection. Placental malaria (PM) often results in severe maternal anemia, spontaneous abortion, pre-term deliveries, intrauterine growth restriction and low birth weight. Despite the serious implications of PM on maternal and child health we know little about the impact of PM on neonatal and infant neurodevelopment. This study examines the impact of in utero exposure to maternal PM infection on the cognitive development of offspring. This study tests the hypothesis that host innate immune response to malaria, specifically generation of activated complement C5-C5a receptor (C5a-C5aR) signaling, mediates adverse offspring outcomes following maternal malaria infection.

In this study we used a mouse model of PM that replicates the pregnancy outcomes and placental pathology of human PM. BALB/c wild type and C5aR-/- dams were infected at gestational day 13 with the rodent malaria parasite, Plasmodium berghei ANKA. The role of the complement system was examined using genetic (C5aR-/-) as well as pharmacological blockade (anti-C5a antibody) of complement activity in dams. Control animals were offspring brought to term by uninfected BALB/c and C5aR-/- dams. Offspring of control and malaria-infected dams were tested in a battery of behavioural tests.

We show that offspring of malaria-infected dams display impairments in learning and memory in the novel object recognition test ($p < 0.005$), and the y-maze test ($p < 0.05$) and anxiety-like behavior in the tail suspension test ($p < 0.01$) compared to offspring from uninfected mothers. Genetic and pharmacological blockade of complement protein C5a signaling in malaria-infected dams rescued the behavioural phenotype observed in offspring in the novel object recognition and tail suspension tests ($p > 0.05$ across both tests). We observed that blockade of C5a signaling in malaria-infected dams rescued the behavioural impairments observed in offspring of PbA-infected dams. Our results show for the 1st time that malaria induced activation of the complement cascade contributes to neurocognitive deficits in offspring from malaria-infected dams.

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Fetal Exposure to the Selective Serotonin Reuptake Inhibitor Sertraline Results in Impaired Fetal Growth and Pancreatic Development in Wistar Rats. Nicole E De Long,[1] Rebecca A Stepita,[1] Valerie H Taylor,[2] Katherine M Morrison,[3] Hertzel C Gerstein,[4] Alison C Holloway.[1] *[1]Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada; [2]Psychiatry, University of Toronto, Toronto, ON, Canada; [3]Pediatrics, McMaster University, Hamilton, ON, Canada; [4]Medicine, McMaster University, Hamilton, ON, Canada.*

Introduction: According to current estimates, 10-15% of women take antidepressant medications during pregnancy. Untreated depression is associated with adverse obstetrical and neonatal outcomes. However clinical studies have also reported that the use of selective serotonin reuptake inhibitor (SSRI) antidepressants during pregnancy is associated with an increased risk of low birth weight. In humans, low birth weight is associated with an increased risk of developing type 2 diabetes in adulthood; a relationship which may reflect abnormal prenatal pancreatic development.The effects of perinatal exposure to SSRIs on pancreatic development, however, have not been examined.

Objective: In this study, we examined the effect of fetal exposure to sertraline (Zoloft®), a SSRI antidepressant, on pregnancy outcomes and pancreatic development.

Methods: Female nulliparous Wistar rats were given vehicle (N=5) or sertraline hydrochloride (SERT 10 mg/kg/d; N=8) via daily subcutaneous injection from the confirmation of mating until parturition. We assessed pregnancy outcomes, serum insulin, glucose and pancreatic development at postnatal day 1.

Results: Sertraline-exposed dams had smaller litters (CON 14.38 ± 0.57 vs SERT, 12.4 ± 0.51; p=0.03) but no increase in the number of stillbirths. Pups born to sertraline-exposed dams had a significantly lower birth weight (CON 6.3 ± 0.2 g vs SERT 5.6 ± 0.2 g; p=0.02) and were more likely to be small-for-gestational age (SGA) relative to the control group (p=0.001). Sertraline-exposed pups had significantly lower serum insulin concentrations (CON 1.1 ± 0.1 ng/ml vs SERT 0.5 ± 0.1 ng/ml, p=0.05) in association with a reduction in the number of pancreatic beta cells (CON 1.1 ± 0.1% vs SERT 0.7 ± 0.1%, p=0.002)

Conclusion: These data demonstrate that in this model fetal exposure to sertraline results in impaired fetal growth and reduced beta cell capacity in the offspring. Since both low birth weight and impaired pancreatic development are risk factors for the development of type 2 diabetes, these results raise concerns regarding the long term metabolic sequelae of fetal exposure to SSRIs.

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Xanthine Oxidase and Programming of Cardiac Dysfunction in Hypoxic Pregnancy: Mechanism and Intervention. Y Niu,[1] AD Kane,[1] CM Lusby,[1] BJ Allison,[1] JJ Kaandorp,[2] JB Derks,[1] DA Giussani.[1] *[1]Physiology, Development and Neuroscience, University of Cambridge; [2]University Medical Center, Utrecht, The Netherlands.*

Introduction: We and others have shown that hypoxic pregnancy programmes cardiac dysfunction (Niu et al. *Reprod Sci.* **17**(3) 2010;A342; Xue et al. *J Pharm Exp Ther.* 2009; **330**: 624; Rueda-Clausen et al. *Card Res.* 2009;**81**: 713). However, the mechanism remains uncertain, preventing targets for intervention. We have also reported that developmental programming of cardiac dysfunction in hypoxic pregnancy is restored by maternal treatment with antioxidants (Niu et al. *Reprod Sci.* **17**(3) 2010; A342; Allison et al. *J DOHaD* 2011;**2(1)**: PI-013), however the source of oxidative stress in hypoxic pregnancy remains unknown. Here, we investigated the role of xanthine oxidase in programming cardiac dysfunction in hypoxic pregnancy.

Methods: Female Wistar rats (n=48) were randomly divided into normoxic (N: 21% O2) or hypoxic (H: 14% O2) pregnancy, with or without maternal treatment with allopurinol (30 mg/Kg in jelly) from days 6-20 of gestation. This experimental model of hypoxia does not affect maternal food intake. At birth, litters were culled to 8 pups (5 males and 3 females) and weighed weekly. At 4 months, following euthanasia, hearts were isolated from 1 male per litter and cardiac function was investigated in a Langendorff preparation.

Results: At 4 months, body weight (528±7 *vs*. 605±2 g, *P*<0.05) but not heart weight was lower in offspring from hypoxic pregnancy compared to controls. Adult offspring from hypoxic pregnancy showed enhanced myocardial contractility (dP/dt max) with increased left ventricular (LV) beta-adrenergic sensitivity, and reduced coronary flow rate (CFR) with impaired recovery from an ischaemic challenge (Fig. 1 A-D). Maternal treatment with allopurinol in hypoxic pregnancy restored all indices of cardiac dysfunction towards control levels in adult offspring.

Conclusions: The data support a link between xanthine oxidase and developmental programming of cardiac dysfunction in hypoxic pregnancy, providing a potential target for intervention.

BHF, The Royal Society, The BBSRC and Internationalization Fund.

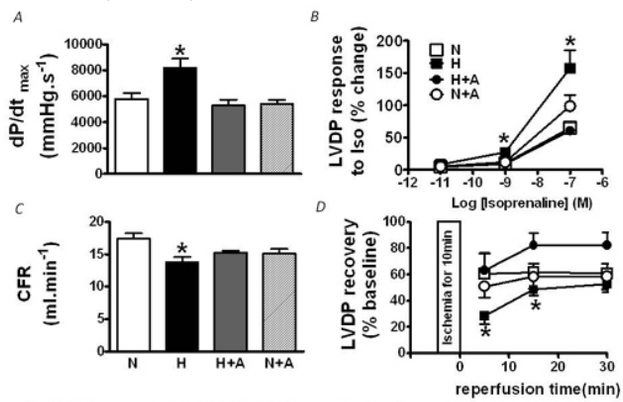

Fig. 1. Data are mean ± S.E.M. dP/dt_{max}, the maximum derivative of the left ventricular pressure; LVDP, left ventricular developed pressure; Iso, isoprenaline. N, n=9; H, n=8; H+A, n=8; N+A, n=9. Significant differences (*P*<0.05) are, for A, B and D: * *vs.* all; for C * *vs.* N. One-Way ANOVA with Tukey Test.

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Activation Status of Maternal Leukocytes and Cytokine Profile Predicts Imminent Preterm Delivery. Sally Sabra,[1,2,3] Oksana Shynlova,[1] Craig Pennell,[4] Stephen Lye.[1,2,3] *[1]Institute of Med Sciences, Univ of Toronto; [2]Samuel Lunenfeld Res Inst, Mt Sinai Hospital, Toronto; [3]Dept Ob/Gyn, Univ of Toronto, Canada; [4]School of Women's & Infants Health, Univ of West Australia.*

Introduction: Up to 70% of women in threatened preterm labour (TPTL) do not deliver within 48h of diagnosis and many subsequently deliver at term. We previously showed that true labour is associated with an influx of maternal

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peripheral immune cells into the myometrium and the induction of a state of physiologic inflammation. We hypothesized that defining the activation status of circulating leukocytes (by FACS and cytokine profiles) might inform the diagnosis of true vs false labour.

Methods: Peripheral blood was collected from women (24-32 weeks of gestation) categorized by labor status: (1) TPTL, delivery within 48h (TrueTPTL, n=10); (2) TPTL with no delivery (FalseTPTL, n=10) and (3) healthy women matched for gestational age (PTNIL, n=28). The activation status of granulocytes (G), monocytes and T-cells was detected using mean fluorescent intensity of specific surface markers (CD55, CD44, and CD181). A panel of 48 cytokines was assessed using Human Cytokine multiplex immunoassays (BioRad) in maternal plasma (n=40) collected from groups (1) & (2).

Results: (1) CD55 (a marker of inflammation) was significantly ($p<0.01$) elevated on T-cells in both TPTL groups as compared to PTNIL, but was not different between FalseTPTL and TrueTPTL groups. (2) CD44 (cell surface glycoprotein involved in cell-cell interactions) was highly up-regulated on T-cells in TrueTPTL vs FalseTPTL ($p<0.01$). (3) CD181 (IL-8 receptor) was elevated on G in TrueTPTL vs FalseTPTL ($p<0.05$); (4) Women in TrueTPTL exhibited significantly higher ($P<0.05$) plasma concentrations of pro-inflammatory mediators IL-6, M-CSF, IL-9, IFNγ, MCP1, TNFα, SCGFβ as compared to FalseTPTL women.

Discussion: Our data suggest that 1) TPTL in general represents an inflammatory state (increased CD44, CD55), 2) those pregnancies that are destined to deliver imminently, in addition, show increased capability for immune cell infiltration (increased CD44) as might be required for transendothelial migration into the myometrium, as well as 3) enhanced responsiveness (increased CD181) to the elevated levels of circulating pro-inflammatory cytokines in trueTPTL. We suggest that efforts to target the early inflammatory events in the labour cascade are more likely to show success in preventing preterm delivery.

Funding: MOD (grant # 21-FY10-204)

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Absence of Maternal CCR2 Reduces the Adverse Effects of LPS-Induced Inflammation on Neonatal Outcome. Bronwen R Herbert,[1] Renyi Hua,[1] Simon N Waddington,[2] Suren R Sooranna,[1] Mark R Johnson.[1] *[1]Surgery & Cancer, Imperial College, IRDB, London, United Kingdom; [2]IfWH, UCL, London, United Kingdom.*

Intrauterine infection/inflammation can cause premature labour (PTL) which accounts for more than 70% of neonatal deaths. Of those neonates that survive, 10% overall and 80% of those born before 26 weeks, have a life-long handicap e.g. cerebral palsy (CP). Infection/inflammation is characterized by chemokine-driven mass infiltration of immune cells into the infected area. In particular, CCL2 which binds to the CCR2 receptor, is secreted by activated astrocytes during central nervous system (CNS) inflammation and recruits monocytes/macrophages and activated lymphocytes into the CNS where it can increase brain endothelial permeability. We hypothesize that a reduction of CCR2 may reduce the harmful effects of maternal infection on the neonatal brain.

10μg of LPS was administered by intrauterine injection on day 16 of gestation to both CD1 and CCR2-/- dams (on a CD1 background). Maternal and fetal tissues were collected 3 and 7 hours post injection and at the onset of labour (defined as the birth of the first pup). Neonatal health was evaluated at the time of tissue collection. Ratios of mRNA gene expression CCL2, CCL5, CCL20, CXCL1, CXCL5, IL-1β, IL-6 and TNF-α to normalised to the housekeeping gene, GAPDH were compared at the different time points.

LPS induced PTL in both strains (CD1 12.6h±1.1; CCR2-/- 13.8±0.9 NS) compared to vehicle controls, and resulted in high levels of pup mortality. All the CD1 pups were dead *in utero* by 7 hours after LPS but 95% of the pups in the CCR2-/- mice and in the control groups were alive. In the myometrium, the LPS-induced increase in chemokines and cytokines mRNA levels were similar and evident by 7h post LPS administration. In the brains of the CD1 pups the expression of CCL2, IL-1β and IL-6 were greater when compared to vehicle controls. In contrast, the brains of the pups from the CCR2-/- dams showed no increase in any chemokine or cytokine and actually showed a reduction in IL-6 expression when compared to vehicle controls. A similar pattern was seen in placentas from LPS-treated CCR2-/- dams compared to CD1 dams, where a reduction in CCL2, CCL, CXCL1, IL-1β and IL-6 was found.

Our data shows that the adverse effect of LPS on pup survival was reduced in the CCR2-/- mouse, suggesting that the administration of a CCR2 antagonist may protect against inflammation-induced neonatal brain injury in at risk human pregnancies.

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N, N-Dimethylacetamide (DMA) Prevents Preterm Birth in a Murine Model by Up-Regulating Interleukin 10 (IL-10). Sruthi Sundaram,[1] Charles Ashby,[1] Khushboo Abhichandani,[1] Swapna Munnangi,[1] Sandra E Reznik.[1,2] *[1]Pharmaceutical Sciences, St. John's University, Queens, NY, USA; [2]Pathology and Obstetrics and Gynecology and Women's Health, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, USA.*

Background: The lack of efficacy of anti-inflammatory drugs, antioxidants and other tocolytics in the treatment of preterm delivery (PTD) has intensified investigation of the pathogenesis of infection-associated PTD with particular interest in the inflammatory pathways likely involved. In this study, the tocolytic effects of a widely used industrial solvent, N,N-dimethylacetamide (DMA) on PTD was investigated.

Objective: Our aim was to assess the tocolytic effects of DMA in infection-associated PTB and investigate its mechanism of action.

Methods: At 15 days' gestation C57BL/6 mice were administered lipopolysaccharide (LPS) (50mg/kg) intraperitoneally (ip) to induce PTD. Increasing doses (0.2 (n=7), 0.39 (n=8), 0.78 (n=10), 1.56 (n=6), 3.2mg/kg (n=8)) of DMA were administered intraperitonealy 30 minutes prior to and 10 hours after the LPS injection. The controls received phosphate buffered saline (PBS) in lieu of DMA (n=9). Histological sections of placentas and uteri from both treated and control groups were analyzed for extent of inflammatory infiltrate. Western blot analysis of these tissues for several cytokines was performed.

Results: DMA significantly reduced the incidence of PTD in a dose dependent manner. Pre-treating animals with 1.56 mg/kg DMA before the LPS challenge significantly decreased levels of interleukin (IL)-6, IL-1β and TNF-α in the placental tissues compared to controls ($p<0.05$). Additionally, we detected increased placental levels of the anti-inflammatory cytokine IL-10 ($p<0.05$). Histological analysis of placental and uterine sections revealed a significant decrease in the levels of circulating polymorphonuclear neutrophils ($p<0.05$). The results suggest that DMA is an effective tocolytic and the mechanism of action is related to its anti-inflammatory effects. At the dose (1.56 mg/kg) used for Western blot analysis and histologic studies, no macroscopic abnormalities of the fetus after necropsy or gross behavioral changes of the mother were observed.

Conclusions: In conclusion, DMA is effective in preventing infection-associated PTD. Its mechanism of action is likely related to its anti-inflammatory effects and up-regulation of IL-10.

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Inhibition of AP1 Delays LPS-Induced Preterm Labour and Improves Pup Outcome in the Murine Model. Yun S Lee,[1] David A MacIntyre,[1] Grisha Pirianov,[2] Bronwen Herbert,[1] Vasso Terzidou,[1] Mark R Johnson,[1] Phillip R Bennett.[1] *[1]Surgery and Cancer, Imperial College, London, United Kingdom; [2]Cardiovascular Sciences Research Centre, St Georges University of London, London, United Kingdom.*

Parturition is initiated by a myriad of biochemical events that include the activation of inflammatory pathways leading to the onset of rhythmic, sustained myometrial contractions. Two critical modulating factors of these inflammatory pathways are nuclear factor-kappa B (NFκB) and activator protein 1 (AP-1). Both regulate the expression of downstream pro-inflammatory cytokines such as interleukin 8 (IL-8) and cyclo-oxygenase 2 (COX-2), which in turn stimulate cervical remodelling, rupture of the fetal membranes and the initiation of myometrial contractions. We have recently shown that NFκB and AP-1 are dually activated in the myometrium of an LPS (*Salmonella abortus*) inflammatory-induced murine model of preterm labour (PTL). Here we show that in an alternative LPS (*Escherichia coli*: serotype O111)-induced PTL model, AP-1 is chiefly activated in the myometrium, which results in preterm birth less than 7 hours post-administration. Although NFκB is also activated, the degree of activation was no higher than that seen in PBS injected animals, which delivered at term. To confirm that AP-1 activation alone is sufficient to induce PTL, we treated the NFκB/AP-1 dual activation model with a JNK-inhibitor, D-JNKI (Qbiogene, Alexis, UK), to suppress AP-1 activation. As a result, inflammation-induced PTL was delayed by 10 hours (n=8) and pup survival was improved from 30 % to 85 %. Our results indicate that AP-1 inhibition is sufficient to delay the cascade leading to myometrial contraction in the LPS-induced inflammatory murine model of PTL and may represent a rational target for future therapeutic interventions.

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Bacteria Identified by PCR in Fetal Membranes Following Preterm Labour Associated with Increased Toll Receptor Expression. Dagmar Albar,[1] Cherry Alviani,[2] Kathryn Harris,[1] Nigel Klein,[1] Donald Peebles.[2] *[1]Institute of Child Health, University College London, London, United Kingdom; [2]Institute for Womens Health, University College London, London, United Kingdom.*

Objectives: Bacteria-triggered inflammation has been shown to cause preterm labour. This study aimed to 1) characterise the bacterial species in fetal membranes of very preterm and term deliveries using species specific real-time PCR and 2) to identify whether the presence of bacterial products was associated with an innate immune response via toll-like receptor signalling (TLR).

Methods: 106 women were recruited, of which 77 delivered before 32 weeks gestation. Fetal membranes and placenta were sampled using a sterile procedure following delivery and DNA extracted. Species-specific quantitative PCR was performed for a panel of 7 bacteria identified as being most prevalent in a previous study. TLR2, 3, 4 and 9 expression was quantified in the same samples by qPCR.

Results: samples from women who delivered preterm were more likely to be bacteria positive(78%) compared to those who delivered at term (36%, $p<0.001$). There was also a significant difference between the groups who laboured preterm and those with an indicated preterm delivery (not in labour, 11.1%, $p<0.001$) Bacteria was present in both vaginal and caesarean preterm deliveries, suggesting that the bacteria were not acquired during passage through the vagina. 42% of preterm fetal membranes harvested 2 or more bacterial species compared to only 8% of term samples ($p=0.002$). *Ureaplasma urealyticum*, Group B Streptococcus and, *Peptostreptococcus micros* and *Snethia/Leptrotrichia* species were associated with preterm deliveries, whereas *Fusobacterium nucleatum*, *Ureaplasma parvis* and *Mycoplasma hominis* were also found in term samples. TLR2 expression was significantly increased in both labour ($p=0.03$) and preterm samples where two or more bacterial species were detected ($p=0.007$). No association was seen with TLR 4; however TLR 9 was significantly increased in women in preterm labour compared to women in labour at term ($p=0.002$).

Conclusion: bacterial DNA, often from 2 or more species, can be found in nearly 80% of fetal membranes following preterm birth. Bacterial presence and preterm birth were associated with increased expression of TLR 2 and 9, suggesting an active role for bacteria in initiation of preterm delivery.

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Chemoattractant Activity of Extracts from Uterine and Fetal Membranes Is Not Related to Maternal Serum Progesterone Concentrations and Is Not Required for Labor in Guinea Pigs. N Gomez-Lopez,[1] WC Tong,[2] S Tanaka,[1] O Hajar,[1] DM Olson,[1] MJ Taggart,[2] GN Europe-Finner,[2] BF Mitchell.[1] *[1]OB/GYN, University of Alberta, Canada; [2]ICM, Newcastle University, United Kingdom.*

Objective: Normal labor is accompanied by infiltration of leukocytes into intrauterine tissues. This phenomenon is regulated by chemoattractant activity (CA) of factors produced in these tissues. The function of these leukocytes is currently unresolved but several studies suggest they may be important for regulation of labor onset. Using a guinea pig model of normal and RU486-induced parturition, our objectives were to assess the CA of extracts of uterus and fetal membranes (FM) on a pool of leukocytes obtained from term gestation (>d64; term gestation is 68 ± 0.3d). We hypothesized that the leukocyte CA would be maximal during spontaneous or induced labor.

Methods: Full thickness uterine and FM tissues were isolated from guinea pigs at different gestational stages: late gestation (LG; d50-64; n=11), term gestation not-in-labor (TNIL; > d64; n=5) and term gestation in spontaneous labor (TSL, >d64, n=6). Preterm labor (PTL, n=6) was induced using RU486 (3 mg/kg body weight) administered on d55 and 56. Birth occurred at 47 ± 7h. Controls (CPTL; d51-59; n=5) received vehicle only. Tissue extracts were prepared and maternal peripheral leukocytes isolated using a Ficoll gradient. CA was measured using a validated Boyden chamber assay. Leukocyte numbers and subpopulations were determined using flow cytometry. Maternal serum P4 levels were quantified by EIA. Statistical analyses used Kruskal-Wallis and Mann-Whitney tests with significance at $P<.05$.

Results: CA of uterine and FM extracts was significantly greater for granulocytes and lymphocytes from TSL compare to TNIL or LG animals. Maternal serum P4 remained unchanged between TSL (205 ± 35 ng/mL; n = 6 samples from <24h before delivery) and TNIL (227 ± 21 ng/mL, n = 14) animals. CA of uterine and FM extracts was significantly less for granulocytes and lymphocytes from PTL compare to CPTL and TSL animals.

Conclusion: CA increases in extracts from uterine and FM tissues at the time of term labor. However, this was unrelated to changes in maternal serum progesterone concentrations. In contrast, RU486-induced PTL was accompanied by decreased chemoattractant activity. These data do not support the concept that leukocyte invasion of uterine and FM tissues is a pre-requisite for labor.

Funding: MRC in UK; AIHS and WCHRI in Alberta

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Placental Adaptation to Maternal Obesity: Role of Oxidative Stress in Activation of Autophagy and Cell Death. S Muralimanoharan, A Maloyan, J Mele, Leslie Myatt. *CPNR OB-GYN, UTHSCSA, SA, TX, USA.*

Obesity during pregnancy is associated with maternal complications, poor perinatal outcome and may have developmental programming effects on the offspring. Previous studies from our laboratory show that with increasing BMI there is an overall increase in placental oxidative stress and a shift from protein carbonylation to protein nitration. Oxidative stress has been recently shown to cause autophagy and cell death under various pathological conditions. We hypothesized that the chronic low grade inflammation of obesity may lead to oxidative stress and pathological dysfunction in the placenta. **The aim of this study was to evaluate oxidative stress and cell death in villous tissue of placenta from lean, overweight and obese women**. As there is a marked sexual dimorphism in placental physiology, we focused on evaluation of placentae with a male fetus. Placentae were collected from lean (LN: BMI 19-24), overweight (OW: BMI 25-29) and obese (OB: BMI 30-45) (n=5 each) women after C-section at term prior to labor. The general production of reactive oxygen species (ROS) measured by dichlorofluorescein staining of cryosections was elevated 5-fold in OW and almost 19-fold in the OB group ($p<0.05$). Oxidative stress, measured as H_2O_2 production, was 1.8 fold higher ($p<0.05$) in OB villous tissue homogenate compared to LN and OW. In parallel, the antioxidant Cu-Zn SOD was 3-fold higher in OB compared to OW and LN ($p<0.005$). Prolonged oxidative stress was previously shown to lead to activation of cell death. Indeed, TUNEL staining showed 6-fold increase in the number of apoptotic nuclei in both OW and OB compared to LN ($p<0.05$). The release of cytochrome c from mitochondria measured by Western Blot was significantly increased ($p<0.05$) in OW and OB compared to LN placentas. Surprisingly, however, no difference in caspase-3 activation was detected in OW and OB groups compared to LN. We examined autophagy as a potential compensatory mechanism. We detected a significant increase in accumulation of the autophagy markers, beclin1 ($p<0.01$) and ATG7 ($p<0.004$) in OB compared to LN and OW groups. This was accompanied by a reduction in p62 ($p<0.04$), a marker for accumulation of misfolded proteins, and LC3 cleavage ($p<0.05$) associated with formation of autophagosomes. This study suggests that excessive production of ROS in OW and OB placenta triggers a cascade of pathological events including initiation of cell death and autophagy.

Supported by NIH HD075297

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Preeclampsia Upregulates Human Placental Expression of Angiogenesis-Associated microRNAs (17, 20a and 20b) That Regulate Trophoblast Differentiation and Invasion by Targeting Ephrin B2 and EPHB4. Wen Wang,[1] Hong-hai Zhang,[1] Lin Feng,[1] Jing Zheng,[2] Dong-bao Chen.[1] *[1]Depts of Ob/Gyn and Path, Univ CA, Irvine, CA, USA; [2]Dept of Ob/Gyn, Univ WI-Madison, Madison, WI, USA.*

Introduction: MicroRNAs (miRNAs) are a class of noncoding -21-25 nucleotide RNAs that negatively regulate gene expression *post*-transcriptionally. The Ephrin/EPH system patterns spiral artery remodeling and cytotrophoblast invasion. **Hypothesis**: Preeclampsia upregualtes miRNAs that regulate trophoblast differentiation and invasion. **Methods**: Total placental microRNA was extracted from severe preeclampsia and normotensive deliveries (n=10/group). Differential miRNA expression was analyzed by human miRNA array containing 894 mature miRNAs. Differentially expressed miRNAs were verified by real-time qPCR. Angiogenesis-associated miRNAs were analyzed by online target prediction databases and the predicted targets were verified by luciferase reporter assay. miRNA precursors or antagomirs were transfected into human trophopblast derived HTR-8/SVneo cells. Total RNA was extracted for determining the downstream target genes by real-time qPCR. Four days miRNA *post*-transfection, the cells were stained with cytokeratin 7 for assessing syncytialization. Cell invasion was accessed by Matrigel invasion assay. **Results**: Seven abundant miRNAs differentially expressed in preeclampsia and normal controls were identified by miRNA microarray. Real time qPCR confirmed that three abundant angiogenesis-associated miRNAs, i.e., miR-17, miR-20a and miR-20b, were significantly upregulated in preeclamptic placentas. Luciferase reporter assay verified that Ephrin-B2

and EPHB4 were direct targets of miR-17, 20a and 20b. In HTR-8/SVneo cells, overexpression of miR-20b decreased Ephrin-B2 mRNA expression and inhibition of miR-20b increased EPHB4 mRNA expression. Ephrin-B2 mRNA expression was significantly reduced in preeclamptic placentas compared to normal placentas. Overexpression of miR-17, 20a and 20b resulted in ~40% less syncytiotrophoblast-like multinuclear cells; inhibition of miR-17, 20a and 20b didn't affect cell syncytialization. Inhibition of miR-20a dramatically increased cell invasion. **Conclusion**: These data implicate that preeclampsia upregulated angiogenesis-associated miRNAs (miR-17, 20a and 20b) regulate cytotrophoblast invasion and differentiation by targeting Ephrin-B2 and EPHB4 (RO1 HL70562 & R21HL98746).

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Mechanisms of Hyperglycemia-Induced Alterations in Trophoblast Migration: A Model for the Pathogenesis of Preeclampsia in Diabetes Mellitus. Christina S Han, Melissa J Mulla, Will Schlesinger, Stephen F Thung, Vikki M Abrahams. *Obstetrics, Gynecology, and Reprod. Sci., Yale University, New Haven, CT, USA.*

Objective: Women with diabetes mellitus (DM) are at risk for adverse pregnancy outcomes, like preeclampsia. Impaired migration of trophoblast into the maternal decidua and spiral arteries in early gestation forms a dysfunctional uteroplacental vasculature, contributing to the pathogenesis of preeclampsia. Known mediators of trophoblast migratory dysfunction include excessive production of tissue inhibitors of metalloproteinases (TIMP), and reduced secretion of IL-6 with concomitant reduced phosphorylation (p) of signal transducer and activator of transcription 3 (STAT3). Therefore, the objective of this study was to evaluate the effect of hyperglycemia on first trimester trophoblast migration, and to determine the associated mechanisms by also evaluating the effect of hyperglycemia on trophoblast TIMP-1, TIMP-2, and IL-6 secretion, and STAT3 phosphorylation.

Methods: The first trimester human trophoblast cell line (Sw-71) was treated with media containing glucose at 5 mM (normoglycemia), 10 mM (borderline hyperglycemia), or 25 mM and 50 mM (hyperglycemia). Trophoblast migration was measured after 48 hrs using a two-chamber colorimetric assay. After 72 hrs, culture supernatants were collected and assayed for TIMP-1, TIMP-2, and IL-6 by ELISA. Cell lysates were evaluated for total and pSTAT3 by western blot and semi-quantitative densitometry.

Results: All levels of hyperglycemia (10-50 mM) significantly reduced trophoblast migration in a dose-dependent manner, when compared to normoglycemic conditions (5mM) ($n=3$; $p<0.001$). All levels of hyperglycemia (10-50 mM) also significantly increased trophoblast TIMP-1 (2.5±0.1-fold) and TIMP-2 (1.7±0.2-fold) secretion ($n=3$; $p<0.01$). Trophoblast IL-6 secretion was significantly increased at true hyperglycemic levels (1.9±0.6-fold for 25 mM, 2.3±0.7-fold for 50 mM) when compared to normoglycemic conditions, and this correlated with increased pSTAT3 expression ($n=3$; $p<0.01$).

Conclusion: These findings demonstrate that hyperglycemia limits the migratory capacity of first trimester trophoblast in vitro, and this limitation may be mediated by increased TIMP-1 and TIMP-2 production in the face of excess glucose. Moreover, this inhibition of trophoblast migration functions independently from the IL-6-pSTAT3 pathway. This may provide a novel mechanism for migratory dysfunction in DM-induced preeclampsia.

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PPARγ Regulates sFlt in Mouse and Human Placenta. Lynlee Wolfe,[1] Veronique Tache,[2] Pooja Iyer,[3] Julia Peng,[3] Mana Parast.[3] *[1]Reproductive Medicine, UC San Diego Health System; [2]Reproductive Medicine, University of California, Davis; [3]Pathology, UC San Diego Health System.*

Background and objective: Preeclampsia has been associated with increased circulating levels of anti-angiogenic molecules, including soluble VEGF receptor-1/sFlt, the major source of which is placental syncytiotrophoblast (STB). PPARγ is a nuclear hormone receptor abundantly expressed in trophoblast. We have previously shown that mouse trophoblast stem (mTS) cells lacking PPARγ have a defect in STB differentiation. We have also shown that hypoxia, which inhibits TS differentiation, decreases PPARγ levels in wildtype TS cells. The goal of this study was to evaluate the relationship between PPARγ expression and signaling, and sFlt levels in both mouse and human placenta.

Methods: We used previously-derived wildtype (WT) and PPARγ-null TS cells, differentiated under normoxia or hypoxia (2% oxygen), and treated with 1 μM rosiglitazone (Rosi, a PPARγ agonist) or carrier (DMSO) alone. We then determined the expression of PPARγ and sFlt by both real-time PCR and Western blot. We also used human placental explants, from late first trimester (10-12 weeks). Placental explants were cultured in either physiologic (5% oxygen) or pathologic (1% oxygen) hypoxia, in the presence or absence of Rosi. After 48 hours, the explants were placed in RNA-later for at least 24 hours prior to snap-freezing. RNA was isolated using the mirVana kit (Ambion), and its integrity assessed by a Bioanalyzer (Agilent). Real-time qPCR was performed for membrane-bound Flt, soluble Flt, as well as PPARγ; data was normalized to 18S.

Results: PPARγ-null TS cells showed a 2-3 fold higher level of both membrane-bound and soluble Flt, compared to WT-TS. Hypoxia decreased PPARγ mRNA and protein 2-3 fold in WT-TS cells. Rosi treatment decreased sFlt mRNA 3-fold, only in normoxia, and only in WT and not in PPARγ-null TS cells. In human placental explants, RNA integrity numbers ranged from 7.9-8.9 for the initial (t=0 hrs) samples and 5.8-6.6 for the 48-hour samples. Real-time PCR showed a 2-5 fold decrease in both membrane-bound and soluble Flt with Rosi treatment, only under physiologic hypoxia; pathologic hypoxia decreased PPARγ levels 4-fold.

Conclusions: There is an inverse relationship between PPARγ and sFlt expression. Activation of PPARγ by Rosi decreased sFlt levels in a PPARγ-dependent manner. Based on these results, we propose PPARγ as a potential therapeutic target in preeclampsia.

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PPARγ Activation with Rosiglitazone Improves Fetal Growth and Spiral Artery Remodeling in Diabetic Pregnant Rats. Natalia I Gokina, Erika Linder, Christopher Williams, Gabriela Goloman, Karen Oppenheimer. *Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA.*

Introduction: In pregnancy, peroxisome proliferator-activated receptor γ (PPARγ) plays an essential role in normal placental development, trophoblast invasion and spiral artery transformation. To define the role of PPARγ in maternal vascular remodeling during diabetic pregnancy we: (1) characterized the effect of diabetes on vascular PPARγ expression; (2) studied the effect of rosiglitazone (Rosi) administration during diabetes on reproductive outcome; (3) evaluated spiral artery remodeling after chronic PPARγ activation.

Methods: Diabetes was induced by i.p. injection of 55 mg/kg streptozotocin (STZ) to rats on 2nd day of pregnancy ($n = 6$). Control rats were injected with citrate buffer (CB, $n = 6$). Rosi (5 mg/kg/day) was given to 6 diabetic rats on days 14 -20 of pregnancy. Rats were euthanized at day 20 of pregnancy. The diameters of pre-placental spiral arteries in the nearest proximity to the mesometrial triangle and the length of their widening were measured from microscope images. Maternal and fetal outcomes were determined for all experimental groups. Real time PCR was performed to quantify mRNA levels of PPARγ in mesometrial triangles.

Results: Diabetes results in marked maternal hyperglycemia, decreased fetal and increased placental weights. Administration of Rosi to diabetic rats produced no effect on maternal glucose levels, resorption rate or liter size. Fetal weights (2.01 ± 0.02 g, $n = 79$ vs. 1.92 ± 0.03 g, $n = 95$) were significantly increased and placental weights were decreased (0.593 ± 0.01 vs. 0.48 ± 0.01 g) by Rosi treatment ($P<0.007$). The diameters of spiral arteries were significantly increased by Rosi from 183 ± 5 μm ($n = 118$) to 215 ± 5 μm ($n = 121$); the spiral artery widening was significantly lengthened from 2.83 ± 0.08 to 4.32 ± 0.07 mm ($P<0.001$). Diabetes was associated with a 72 % reduction in PPARγ mRNA expression that was not modified by Rosi administration.

Conclusion: Activation of PPARγ during diabetic pregnancy improves fetal growth in part due to restoring deficient spiral artery transformation. Diabetes-reduced expression of PPARγ in trophoblasts is most likely responsible for insufficient remodeling of spiral arteries. Activators of PPARγ may be useful therapeutic tools for treatment of fetal complications in gestational diabetes or preeclampsia.

Supported by NIH HL088245

O-172

Regulation of Trophoblast Amino Acid Transporter Trafficking by mTOR Complex 1 Is Mediated by the Ubiquitin Ligase Nedd4-2. Fredrick J Rosario, Theresa Powell, Thomas Jansson. *Center for Pregnancy and Newborn Research, Dept OB/GYN, Univ of Texas Health Sci Center, San Antonio, TX, USA.*

Introduction: The mammalian target of rapamycin (mTOR) is a protein kinase that responds to nutrient availability and growth factor signaling to control cell growth. mTOR exists in two complexes, mTORC1 and mTORC2. We have previously shown that silencing of mTORC1 or mTORC2 inhibits trophoblast System A and L amino acid transport activity by affecting transporter trafficking to the plasma membrane. However, the molecular mechanisms by which mTOR

regulates the cell surface expression of trophoblast amino acid transporters is unknown. Nedd4-2 is an ubiquitin ligase that catalyzes the ubiquitination of proteins localized in the plasma membrane. Because ubiquitination is recognized as a signal to target a protein for internalization, Nedd4-2 controls cell surface expression of proteins such as nutrient transporters. We tested the hypothesis that Nedd4-2 mediates the regulation of amino acid transporter membrane trafficking by mTOR. **Methods:**Human primary cytotrophoblast cells isolated from (n=3-5) normal term placentas were cultured for 18 hrs and then transfected with siRNA targeting raptor (silences mTORC1), rictor (silences mTORC2), Nedd4-2, raptor plus Nedd4-2, rictor plus Nedd4-2 or scrambled siRNA (control). Cells were allowed to syncytialize in culture and at 90 hours, System A activity was measured as Na^+dependent ^{14}C-methyl aminoisobutyric acid uptake, and System L amino acid transporter activity was determined as 2-amino-2-norbornanecarboxylic acid inhibitable uptake of ^{3}H-leucine.**Results:**Transfection of primary human trophoblast cells with raptor siRNA resulted in a 50% decrease ($p<0.05$) in raptor protein expression and transfection with rictor siRNA resulted in 55% knock-down ($p<0.05$) of rictor protein levels. Furthermore, transfection with Nedd4-2 siRNA caused a 70% knock-down ($p<0.01$) of Nedd4-2 protein levels. Raptor or rictor silencing significantly inhibited both System A and L amino acid uptake, as shown previously.Nedd4-2 silencing markedly increased both System A (+158 ± 58%, $p<0.04$, n=5) and L uptake (+93 ± 32%, $p<0.05$, n=5) compared to control cells.When combined, the silencing of Nedd4-2 attenuated the decrease in Systems A and L uptake in response to raptor, but not rictor silencing (n=3). **Conclusion:**Our data suggests Nedd4-2 mediates the regulation of trophoblast amino acid transporter membrane trafficking through mTORC1, but not mTORC2.

T-001

Offspring (OFF) Sperm Function Is Impaired by Maternal Obesity (MO): Influence of Oxidative Stress (OS) and Pre-Pregnancy Dietary Intervention (DINT). Elena Zambrano,[1] Claudia Vega-Garcia,[1] Lourdes Boeck,[1] Luis Reyes-Castro,[1] Peter W Nathanielsz,[2] Fernando Larrea.[1] *[1]Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición, Mexico City, Mexico; [2]OB/GYN, UTHSCSA, San Antonio, TX, USA.*

INTRODUCTION: MO predisposes OFF to metabolic , cardiovascular and endocrine disease. However, effects on reproductive performance are limited. We hypothesized that MO impairs OFF sperm and this is reversed by our model of DINT(1).

METHODS: We studied Wistar rats fed weaning through pregnancy and lactation on chow (C) or high-energy obesogenic diet (MO). A third group switched from MO to C at postnatal day (PND) 90 (DINT). Mothers bred PND 120 and ate pregnancy diet until weaning. OFF ate C from weaning. Six male OFF (different litters) were euthanized at PND 450. Fat depots were weighed. Adiposity index (AI): (thoracic and visceral fat wt/wt body) * 100. Sperm was obtained from epididymal tail and efferent duct. Sperm qualitywas tested. Sperm OS was evaluated with malondialdehyde (MAD) by spectrometry, and superoxide dismutase (SOD) activity by xanthinoxidase assay and glutathione peroxidase (GPx) by chemical colorimetry. One-way ANOVA; $p < 0.05$. Different letters different $p < 0.05$..

RESULTS: At PND 450 OFF weight was C: 547 ± 25a, MO: 627 ± 13b and DINT: 563 ± 9a (g), gonadal fat C: 12 ± 0.4a, MO: 16.5 ± 1.3b, DINT: 14 ± 0.3ab (g), and AI, C: 6.0 ± 0.3a, MO: 8.2 ± 0.5b and DINT 6.0 ± 0.2a. MO OFF sperm viability, motility, concentration and fertility rate (FR) were all decreased (Fig 1A-D). DINT restored FR to normal with intermediate effects on viability, motility and sperm concentration (Fig 1A-D). MDA was significantly increased in both MO and DINT OFF (Fig 1E). GPx and SOD activities were decreased in MO and DINT.

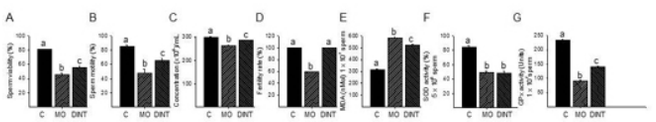


Figure 1.

Fig 1 Sperm nd reproductive characteristics. M ± SEM; n=6. Statistics and abbreviations as in text.

CONCLUSION: MO decreases OFF sperm viability, motility and fertility associated with increased OS and gonadal fat and reduced sperm anti-oxidant enzymes. DINT recuperated gonadal fat and the functional end point of FR.

[1] Zambrano E, et al. (2010). J Physiol; 588 :1791.

T-002

AHR Signaling and Induction of Free Radical Scavengers in Spermatocytes Exposed to Cigarette Smoke. Kenan Omurtag, Praba Esakkay, Deborah Hansen, Kelle Moley. *Obstetrics and Gynecology, Washington University-St Louis, St Louis, MO, USA.*

Background

The toxic effects of cigarette smoke are mediated by the arylhydrocarbon receptor (AhR) by inducing free radical scavenger genes. Considerable epidemiologic research has examined the association between polyaromatic compounds and male infertility, however the effect of AhR activation and its dowstream effects in male germ cells is not fully understood. Here, we show the effect of cigarette smoke on the male reproductive tract through expression of AhR, its chaperone protein HSP90, and the expression of certain target genes.

Methods

C57B6 male mice (n=9) injected daily with 40 µg/mL cigarette smoke condensate (CSC) or control for one week were performed x3. Testes were frozen and cell types isolated using laser microdissection. QRT PCR was performed on these samples for HSP90, SOD2, CYP1A1 and GPX4. Murine spermatocytes (GC-2spd, ATCC) were treated with CSC (100ug/ml) or control with analysis at different time points (12-48 h). QRT-PCR and Western blot were performed to quanlify expression of HSP90, AHR, SOD2 and CYP1A1. CSC treated GC-2spd cells were immunolocalized to determine CYP1A1 expression. Mice were housed according to IACUC and NIH guidelines.

Results

In-vivo expression of spermatocyte derived GPX4 and SOD2 mRNA showed a significant increase compared to controls (1.5 fold, p<]0.05; 2.72 fold increase, p<0.01 respectively). In-vitro expression of HSP90 (3.5 fold, p<0.01), AHR (2.5 fold, p<0.01), SOD2 (1.5 fold, p<0.05) and CYP1A1 (5.5 fold, p<0.001) mRNA were all increased in cells exposed to CSC compared to controls at 12 hours. CYP1A1 showed the greatest effect with 5.5-fold difference in mRNA expression, but only marginal induction on immunoflourescence. Accordingly, spermatocytes exposed to CSC showed greater (2 fold) protein expression at 18 and 24 hours for HSP90, but SOD2 and AhR did not show significant difference compared to the untreated groups.

Conclusion

Male gametes exposed to cigarette smoke have shown an association with increased free radical scavenger induction and decreased sperm concentration and morphology which could impair fertilization. We describe how cigarette smoke induces oxidative stress within 24 hours of exposure in-vitro and in-vivo after a week long exposure. Future in-vivo studies will examine the reproductive effects of male mice exposed to cigarette smoke and/or dioxins to further elucidate the reproductive outcomes of xenobiotic AhR agonists.

T-003

Gonadal Function Recovery in 203 Very Long-Term Male Survivors of Childhood Cancer Using Paired Inhibin B Levels. Wendy van Dorp,[1,2] Ivana MM van der Geest,[2] Marry M van den Heuvel-Eibrink,[2] Sebastian JCMM Neggers,[3] Andrica CH de Vries,[2] Rob Pieters,[2] Joop SE Laven.[1] *[1]Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Pediatric Oncology/Hematology, Erasmus Medical Center-Sophia's Children's Hospital Rotterdam, Rotterdam, Netherlands; [3]Internal Medicine, Section Endocrinology, Erasmus-MC University Medical Center Rotterdam, Rotterdam, Netherlands.*

Introduction: Significant improvements in childhood cancer survival rates over recent decades have increased the importance of long-term treatment effects, such as gonadal dysfunction. Gonadal function in men can be screened using the reliable new serum marker Inhibin B, which can thus be used to identify risk groups for impaired gonadal function in childhood cancer survivors. To date, there have been no large long-term follow-up studies in such survivors, and Inhibin B has not been used as a fertility marker during follow-up.

Aims: To use paired Inhibin B levels to study recovery of gonadal function in very long-term male survivors of childhood cancer.

Methods: In this retrospective single-center study we studied Inhibin B levels in 203 adult survivors at two time-points, and used a logistic predictive model to determine their chance of achieving normal Inhibin B serum levels.

Results: Men had ended treatment at a median age of 8.2 years (range 0.0-20.8) and had first been screened after a median of 15.7 years (range 3.0-37.0). Median interval between the two assessments was 3.3 years (range 0.7-11.3). Median Inhibin B level of all survivors was 127 ng/L (range 5-366 ng/L) at first assessment and 155 ng/L (range 10-507 ng/L) at second assessment. The prediction model suggests that Inhibin B levels do not recover in survivors with a first Inhibin B level below 80 ng/L, which included mainly survivors of Hodgkin's lymphoma treated with metchlorethamine, vincristine, procarbazine and prednisone (MOPP) and rhabdomyosarcoma or survivors treated with pelvic irradiation or AAD scores ≥ 3.

CONCLUSIONS: Long after treatment ended, survivors whose Inhibin B levels were already low at first assessment do not seem to recover.

T-004

Voluntary Exercise (Ex) Reverses Increases in Oxidative Stress (OS) and Impaired Sperm Function in Offspring (OFF) of Obese Rats. Claudia Vega-Garcia,[1] L Boeck,[1] Luis Reyes-Castro,[1] Magaly Vazquez,[1] Fabiola Cruz-Perez,[1] Peter W Nathanielsz,[2] F Larrea,[1] Elena Zambrano.[1] *[1]Departamento de Biologia de la Reproduccion, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; [2]OB/GYN, UTHSCSA, San Antonio, TX, USA.*

INTRODUCTION: Adverse OFF metabolic outcomes due to maternal obesity (MO) are well documented but effects on OFF reproduction are limited. We observed increased ROS and impaired sperm function in OFF of MO rats. There is a need for interventions designed to determine whether developmental programming outcomes are permanent or reversable by OFF life-style modification. We hypothesized that regular OFF Ex reverses adverse effects of MO on OFF sperm quality.

METHODS: We fed female Wistar rats from weaning through to lactation on chow (C) or high energy, obesogenic diet (MO). Mothers were bred at PND 120 and ate pregnancy diet until weaning. OFF ate C from weaning. Six male OFF (different litters) wheel-ran 15 min, 5 times/ week from PND 330 to 450, euthanized at PND 450, fat depots weighed, epididymal sperm obtained to test quality (viability, motility, concentration). Sperm OS was measured by malondialdehyde (MAD - spectrometry), and superoxide dismutase (SOD -

activity by xanthinoxidase assay) and glutathione peroxidase (GPx -chemical colorimetry). Statistics Two way ANOVA (maternal diet and OFFex).
RESULTS: PND 450 OFF wt - C: 547 ± 25a, C+E: 484 ± 14b, MO: 627 ± 13c and MO+E: 586 ± 12ac (g), gonadal fat C: 13 ± 0.4a, MO: 17 ± 1.3b, C+E: 11 ± 0.6a and MOex: 13 ± 1.3a (g). MO OFF sperm viability, motility, concentration, fertility rate (FR) and GPx and SOD activities decreased (Fig 1) and MDA increased in MO OFF. MOEx restored all variables (Fig 1).
CONCLUSION: MO decreases OFF sperm viability, motility and fertility associated with increased OS and gonadal fat, and decreased sperm anti-oxidant enzymes. OFFEx recuperates all sperm variables including gonadal fat and the functional end point of FR. HD21350

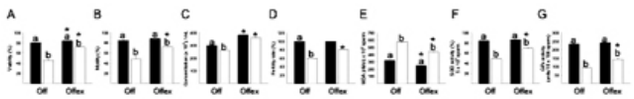
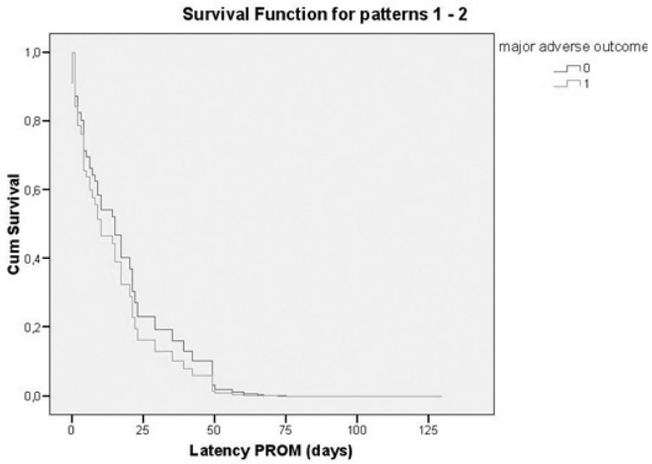


Fig 1 OFF of C (solid) and MO (open) rats. OFF – no exercise, OffEx - exercised OFF. Data M ± SEM, Different letters differ C vs MO, * OFF vs OFFex, $p < 0.05$.

T-005

Is Latency a Risk Factor for Major Adverse Infant Outcomes in Preterm Premature Rupture of Membranes? Federica Accordino,[1] Sara Consonni,[1] Francesca Moltrasio,[3] Tiziana Fedeli,[2] Gaia Kullman,[4] Serena Mussi,[1] Anna Locatelli.[1] *[1]Obstetrics and Gynecology, University of Milano-Bicocca, Monza, Italy; [2]Neonatology, University of Milano-Bicocca, Monza, Italy; [3]Pathology, University of Milano-Bicocca, Monza, Italy; [4]Neuropsychiatry, S.Gerardo Hospital, Monza, Italy.*
Objective:Conservative management in pPROM is controversial. We hypothesized that,when controlled for gestational age (GA) at pPROM,prolonged exposure to intrauterine environment is not a risk factor for composite poor neonatal and infant outcome.
Methods:We studied a cohort of 109 babies born <34 weeks from 01-2006 to 06-2010 from singleton pregnancies complicated by pPROM.Neonates fulfilling inclusion criteria were admitted to neurodevelopmental (ND) follow-up.Excluded were pregnancies with other complications (i.e.preeclampsia). We related obstetric factors and placental histology to major adverse outcome represented by neonatal death (<28 days) or neurodevelopmental injuries (cerebral palsy,mental retardation,neurosensitive damage).Statistical analysis included Chi-square,ANOVA and Cox regression,with P<0.05 considered significant.
Results:pPROM occurred at 26.6±5.7 weeks.Mean latency was 20.8 days±30.8. 13 infants (12%) died and neurodevelopmental follow up was available in 65/76 infants (85%).Adverse ND outcome occurred in 23.1% (n=15/65), and overall major adverse outcome occurred in 28/78 cases (36%).GA at pPROM (22.3±5.4w vs 27.5±4.8w,p<0.001),GA at delivery (27.5±3.2w vs 29.9±2.4w,p< 0.001) and birth-weight (1,115±505g vs 1,403±384g,p=0.008) were significantly lower in cases with major adverse outcome, while latency was longer (36.1±33.9d vs 18.4±29.7d,p=0.019).Antenatal corticosteroids (23/28,82.1% vs 47/50,94%,p=0.127),oligohydramnios (16/28,57.1% vs 17/50,34%,p=0.058) and histologic chorioamnionitis (18/28,64.2% vs 21/50,42%,p=0.098) were not significantly related to major adverse outcome. Cox Regression for Survival showed that latency did not independently predict major adverse outcome after adjusting for GA at pPROM (HR 0.804,95% CI 0.470-1.376).



Discussion:In pPROM latency is not a risk factor for major adverse outcome in infants born at <34 weeks.

T-006

Dystocia in Labour: Maternal of Fetal Problem? P Alimondi,[1] SF Deiana,[3] X Santopietro,[2] C Mastromatteo,[2] PM Villa,[2] E Gianbattista,[2] A Padoan,[2] A Tampieri,[2] A Perino,[1] DE Rinaldo,[4] FA Ragusa.[2] *[1]Ob/Gyn Dept, Palermo; [2]Ob/Gyn Dept, ICP Sesto San Giovanni; [3]Ob/Gyn Dept, Cagliari; [4]Ob/Gyn Dept, Seriate.*
AIM OF THE STUDY
We conducted this study to understand if there is a relationship between fetal position (both head and spine) and dynamic and mechanical dystocias.
We evaluated fetal position in cases of dynamical and mechanical dystocias during first and second stage of labour.
MATHERIALS AND METHODS
This was a prospective observational study performed in Sesto S. Giovanni, Milan,Obstetrical Dept. during a 6 months period. Singleton uneventful term pregnancies (>37 wks) with cephalic presentation were enrolled. Women planned for an elective CS were excluded.
We recorded all cases of dynamic and mechanical dystocia during first and second stage of labour.
We evaluated fetal head (OP/OA) and spine (SP/SA) position both clinically and by serial intrapartum transabdominal ultrasound at the beginning of labour and every 2 hours until delivery.
RESULTS
The ones presented are preliminary data from the ongoing study.
Between January and June 2011 we had 500 deliveries: 68 women, planned for an elective CS were excluded. We recorded 16 cases of dystocia (3,7% on 432 deliveries).
In 35% of cases fetal position was OA/SA, in 25% was OP/SA, in 40% was OP/SP.
When the fetus had a posterior spine, irrespective of OA/OP position of the head, in 71% an urgent CS was performed and the remaining 29% of cases underwent a vaginal operative delivery performed by skilled consultants. No fetus was born without intervention.
DISCUSSION
Our data confirm that a fetal posterior position is the major cause of dynamic and mechanical dystocia, occurring in 65% of cases.
Moreover we observed that the position of the fetal spine is more relevant since dystocias with a SP are more difficult to deal with, regardless of fetal head position.
Dystocias with OP/SP fetus were the most difficult to treat, representing a true obstetric challenge: none of these pregnancies had a spontaneous vaginal delivery, they all required an operative delivery (abdominal or assisted).
Intrapartum ultrasound allows to assess fetal head and spine position and their change throughout labour and delivery and to diagnose fetal malposition, one of the most common causes of dystocia.
The detection of fetal posterior spine has implications for clinical practice: it is important to identify those pregnancies that will require an instrumental delivery by a skilled obstetrician.

T-007

Outcome of Induction of Labor in Relation to Clinical Indication: Retrospective Analysis of 1721 Patients. Maria C Autuori,[1] Laura Avagliano,[1] Patrizia Bozzetti,[1] Alberto Morabito,[2] Mona Mansour,[1] Anna M Marconi.[1] *[1]Unit of Obstetrics and Gynecology, San Paolo Hospital, Milan, Italy; [2]Unit of Statistics, San Paolo Hospital, Milan, Italy.*

Objective: to analyze the obstetric outcome of women undergone induction of labor in relation to the clinical indication and parity.

Methods: we analyzed 1721 women, 1192 (69.3%) nulliparous and 529 (30.7%) multiparous who underwent pharmacological or mechanical induction of labor. We divided these women in 8 subgroups according to the clinical indication for induction of labor: oligohydramnios, premature rupture of membranes, post-date pregnancy, gestational or pregestational maternal disease, intrauterine growth restriction, non reassuring CTG, macrosomia and others.

For each clinical indication we analyzed the modality of delivery and the time interval to vaginal delivery in relation to parity and the neonatal outcome.

Results: vaginal delivery after induction was achieved in 83.6% (1438/1721) women, with a significant difference between nulliparous and multiparous: 78.1% (931/1192) vs 95.8% (507/529) (p=0.001). In two groups the rate of cesarean section was particulary high: non reassuring cardiotocography trace 23.3% (14/60) and macrosomia 24.6% (14/57). In the latter all cesarean sections, except one, were made in the group of nulliparous patients.

The interval period to vaginal delivery was 879±454 minutes and 638±424 minutes (mean ± standard deviation) (p=0.001) respectively in nulliparous and multiparous women; 54 % (407/931) of nulliparous and 68.4% (347/507) of multiparous women delivered within 12 hours (p=0.001).

For all indications, except for the groups of macrosomia and others, the rate of multiparous women who delivered within 12 hours was significantly higher.

For both nulliparous and multiparous women, the categories non reassuring CTG and PROM had a short interval period whereas macrosomia and IUGR were the groups who had longer time interval to vaginal delivery.

Conclusions: multiparous women responded better than nulliparous to induction of labor both in term of rate of vaginal delivery and induction interval time for each clinical indication. Macrosomia was the category which answered worse to induction of labor both in terms of higher cesarean section rate and longer interval time to vaginal delivery.

T-008

Cervical Surgery Is a Risk Factor for Preterm Birth in Nulliparous Patients with Preterm Labor. Jamie A Bastek, Amy L Turitz, Sindhu K Srinivas, Meghan A McShea, Markley N Foreman, Michal A Elovitz. *Maternal and Child Health Research Program, Department of Obstetrics and Gynecology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.*

OBJECTIVE: Existing data is inconclusive regarding whether a history of cervical surgery (CxSurg) including LEEP or cold knife cone biopsy predisposes women to an increased risk of preterm birth (PTB). Whether women with this history may be at increased risk of PTB in the setting of preterm labor (PTL) has not been studied. Therefore, our objective was to determine whether CxSurg increases the risk of PTB in women with PTL.

STUDY DESIGN: Analyses were conducted as part of a larger, prospective cohort study of women with singleton pregnancies enrolled 22-33 6/7 weeks due to symptoms of PTL (4/09 - 12/10). Pertinent maternal information including history of CxSurg and obstetric history (nulliparous, prior PTB, prior full term birth only) was obtained. Significant associations between categorical variables were determined with chi-square analyses. Tests of homogeneity were performed to assess for effect modification and relative risks of PTB were calculated.

RESULTS: 595 women were enrolled for which delivery information was available on 95.5%. The overall rate of PTB <37 weeks was 38.9%. The prevalence of PTB was significantly different by obstetric history (nulliparous: 30.2%, prior full term birth only: 36.1%, prior PTB: 55.7%, p<0.001). Overall, 3.7% (N=22) of patients had CxSurg. Obstetric history was an effect modifier of the relationship between CxSurg and PTB (p<0.0001). In nulliparous women, CxSurg increased the risk of PTB 3.4-fold (RR 3.43, 95%CI 2.64-4.45) compared to women without CxSurg. In multiparous patients, CxSurg increased the risk of PTB in women with prior full term birth only (RR 1.70, 95%CI 1.01-2.89) but not in women with a prior PTB (RR 1.13, 95%CI 0.65-1.99). History of abnormal pap smear was not significantly associated with risk of PTB (p=0.76).

CONCLUSION: In nulliparous women, prior CxSurg significantly increases the risk for PTB. This information should be considered when triaging nulliparous patients presenting with PTL. Whether these women are candidates for screening strategies, such as cervical length, requires further investigation.

Funding: MOD#21FY08-539 (Elovitz); K12HD001265 (PI Driscoll; Scholar Bastek)

T-009

Pharmacodynamic Impact of 17-hydroxyprogesterone Caproate (17-OHPC) in Singleton Gestation. Steve N Caritis,[1,3] Raman Venkataramanan.[2] *[1]Obstetrics and Gynecology, University of Pittsburgh; [2]Pharmaceutical Sciences, University of Pittsburgh; [3]MFMU Network, the Eunice Kennedy Shriver NICHD.*

Background

We have reported an inverse relationship between 17-OHPC concentration and gestational age(GA) at delivery in women with twin gestations receiving 17-OHPC. This study evaluates the relationship between 17-OHPC and GA at delivery in women with singleton gestations.

Methods

This is a secondary analysis of a randomized, double masked, placebo-controlled trial evaluating efficacy of omega 3 supplementation in reducing recurrent preterm birth. Women with singleton gestation were assigned to daily omega-3 supplement or matching placebo from 16–22 through 36 weeks of gestation. All participants received 250 mg 17-OHPC IM weekly from 16- 36 weeks, inclusive. Blood obtained at 25-28 weeks was analyzed for 17-OHPC via LC-MS-MS with a limit of detection of 1ng/ml. This case control study included women who received all scheduled 17-OHPC injections. Cases delivered < 37 weeks and controls delivered ≥ 37 weeks.

Results

Omega 3 treatment did not affect 17-OHPC concentrations or GA at delivery; therefore, cases and controls were selected without regard to treatment group. Table 1 summarizes demographic variables and concentrations of progesterone and 17-OHPC in the two groups.

Table 1	Delivery <37 wks	Delivery ≥37 wks	p value
BMI,mean (SD)	26 (7)	26 (7)	0.740
Race, N (%)			
African American	31 (29)	56 (27)	0.021
Hispanic	7 (7)	38 (18)	
White	68 (64)	116 (55)	
GA at sample,mean (SD)	27 (1)	27 (2)	0.732
Progesterone (ng/ml), mean (SD)	70 (28)	72 (23)	0.216
17-OHPC (ng/ml),mean (SD)	9.9 (4)	10.9 (5)	0.054

Neither progesterone or 17-OHPC concentrations differed significantly in the two groups. After adjusting for race, BMI, treatment group and GA at sampling, no significant association was observed between 17-OHPC concentration and either preterm or spontaneous preterm delivery. A non-significant trend of decreased odds of preterm delivery was seen with higher 17-OHPC concentration.

Table 2	PTB	sPTB
Per unit increase in 17-OHPC	0.95 (0.90-1.01)	0.95 (0.89-1.01)
Quartile- 17-OHPC		
1 (lowest)	1.00 (reference)	1.00 (reference)
2	0.60 (0.31-1.19)	0.68 (0.34-1.37)
3	0.53 (0.27-1.06)	0.61 (0.30-1.22)
4	0.54 (0.27-1.08)	0.51 (0.25-1.05)

Conclusions

Plasma concentrations of 17-OHPC were not statistically different in women who deliver at preterm vs term. We could not demonstrate a significant relationship between 17-OHPC concentrations and risk of preterm birth.

T-010

Epidemiology of Magnesium Sulfate Use in Preterm Gestations Delivered from 24 to 32 Weeks at a University Center. Brendan D Connealy, Carlos A Carreno, Benjamin A Kase, Sean C Blackwell. *Obstetrics, Gynecology, and Reproductive Sciences, University of Texas Health Science Center, Houston, TX, USA.*

Objective:

Exposure to magnesium sulfate before 32 weeks reduces the risk of moderate to severe cerebral palsy in neonates. We intend to describe the contemporary use of magnesium sulfate in patients delivered before 32 weeks based on the indication for administration and the preterm birth (PTB) subtype.

Study Design:

All PTBs delivered between 24 wks 0 days and 31 wks 6 days at a university center from Jan 1 to Dec 31, 2010 were included in the study. Exclusion criteria included stillbirth, major congenital anomalies and multiple gestations. PTBs were categorized based on the indication for magnesium sulfate exposure as

tocolysis, seizure prophylaxis, neuroprotection or no exposure as documented in the medical record and this exposure was further assessed based on the following PTB subtypes; spontaneous preterm labor (sPTL), preterm premature rupture of membranes (PPROM), and medically indicated (MI).

Results:

Over the study period there were 5082 total births and 177 (3.5%) delivered between 24 wks 0 days and 31 wks 6 days. 141 patients met inclusion criteria. The frequency of magnesium sulfate usage based on indication is shown in table 1. 30% of the patients received magnesium sulfate for neuroprotection and 106 patients (75%) were exposed to magnesium sulfate for differing indications.

Frequency of magnesium sulfate exposure based on indication (n=141).

Indication	Number exposed (%)
Tocolysis	14 (10%)
Neuroprotection	43 (30%)
Seizure prophylaxis	49 (35%)
No exposure	35 (25%)

Nearly two thirds (64%) of patients with PTB due to PPROM received magnesium sulfate for neuroprotection. 74% of patients with medically indicated PTB received magnesium sulfate for documented seizure prophylaxis.

Frequency of exposure to magnesium sulfate based on indication and PTB subtype

	PTB Subtype		
Magnesium indication	sPTL (n=43)	PPROM (n=36)	MI (n=62)
Tocolysis	12(28%)	2(5.5%)	0(0%)
Neuroprotection	15(35%)	23(64%)	5(8%)
Seizure prophylaxis	1(2%)	2(5.5%)	46(74%)
No exposure	15(35%)	9(25%)	11(18%)

Conclusion:

Only 30% of the patients had neuroprotection as the indication for magnesium sulfate use documented in the medical record despite the 2010 ACOG and SMFM joint statement supporting magnesium for neuroprotection. 75% of patients with PTB < 32 weeks in our study were exposed to magnesium sulfate for other indications with potential neuroprotective benefit.

T-011

Single Embryo Transfer (SET) during In Vitro Fertilization (IVF) Does Not Significantly Reduce the Risk of Preterm Delivery. Kelecia R Brown,[1] Ndidiamaka Onwubalili,[2] Adam J Fechner,[2] Sangita K Jindal,[3] Laura T Goldsmith,[2] Peter G McGovern.[2] *[1]Obstetrics and Gynecology, Long Island Jewish Medical Center, New Hyde Park, NY, USA; [2]Obstetrics, Gynecology and Women's Health, UMDNJ-New Jersey Medical School, Newark, NJ, USA; [3]Obstetrics and Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, NY, USA.*

Despite efforts to reduce the incidence of preterm delivery in the United States, more than 12% of pregnancies still result in delivery prior to 37 weeks gestation. It has previously been established that the use of controlled ovarian hyperstimulation (COH) and IVF increase the risk of preterm delivery, in part because of the relatively high incidence of multiple gestations. To address this problem, many advocate performing SET to reduce the risk of twins or higher order multiples to a level consistent with the general population. However, the risk of preterm delivery following IVF persists even when controlling for multiple gestations. In exploring this phenomenon, we have demonstrated that luteal mass as well as serum relaxin levels correlate with preterm delivery in singleton pregnancies, suggesting that the risk of prematurity may be due more to the COH rather than the number of embryos transferred. Here we test our hypothesis that incidence of preterm delivery following COH-IVF combined with SET remains significantly elevated above the baseline population risk.

Data on birth outcomes following fresh, non-donor SET cycles from 2008-2009 were obtained from the Society for Assisted Reproductive Technologies. A total of 3151 cycles met criteria, resulting in 1526 livebirths (48.4%). Of these pregnancies, twenty-seven resulted in twins (1.8%) and one resulted in triplets (0.06%). The overall preterm delivery rate was 19.6%, with a distribution of gestational age at delivery as detailed below (Table 1).

Table 1: Distribution of Gestational Age at Delivery following SET

Gestational Age at Delivery (weeks)	Number (%)
12-20	38 (2.4)
20-32	57 (3.6)
32-37	215 (13.6)
>37	1272 (80.4)

These data demonstrate that the increased risk of prematurity persists despite the use of SET and further supports our hypothesis that another aspect of IVF, rather than multiple gestations, is in large part responsible for the increased rate of preterm delivery.

T-012

Use of Magnesium Sulfate for Neuroprotection for Women in Preterm Labor in New Jersey. Janelle Foroutan,[1] Valeria DiStefano,[2] Jacob Kowenski,[2] Aiyanna Burton,[2] Revital Faro,[2] Cande V Ananth,[2] Todd Rosen.[2] *[1]Obstetrics and Gynecology, St. Lukes-Roosevelt Hospital, New York, NY, USA; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson University Hospital, New Brunswick, NJ, USA.*

Objective: Multiple randomized controlled trials have established the antenatal benefits of magnesium sulfate (MgSO4) for reducing the risk of cerebral palsy in surviving preterm infants. This study sought to determine the rate of use of MgSO4 for women at risk for preterm delivery in New Jersey hospitals as well as which factors influence obstetricians to incorporate new research into clinical practice.

Study Design: A cross-sectional anonymous telephone survey was conducted between November 2009-January 2010 with the chief of obstetrics, director of MFM, and charge nurse at all 52 NJ hospitals with a labor and delivery to investigate knowledge and practice patterns pertaining to the management of women in preterm labor. A follow-up survey was performed in July 2011, 1 year after the publication of the ACOG committee opinion endorsing the use of MgSO4 for women at risk for preterm birth.

Methods: Statistical analysis included responses from 112 physicians and nurses with an overall response rate of 79%. 21% of hospitals surveyed reported use of MgSO4 for neonatal neuroprotection prior to ACOG recommendations. 63% of respondents reported use of progesterone and 100% reported use of antenatal steroids, following evidence-based recommendations for women at risk for preterm delivery. 100% of hospitals reported using bedrest and tocolysis in at risk women. Of the respondents not using MgSO4 for neuroprotection, 56% were aware of the evidence-based neuroprotective benefits. Respondents not currently using MgSO4 stated they would change their practice if recommended to do so by ACOG. Subsequent to the completion of the initial survey, ACOG published a committee opinion supporting the use of MgSO4 before anticipated preterm birth to reduce the risk of cerebral palsy in surviving infants. More than one year after this publication, 52% of hospitals were using MgSO4, a 148% increase.

Conclusion: Despite well documented evidence of the benefits of MgSO4 when given to women at risk of preterm birth, most obstetricians in NJ hospitals began using MgSO4 for neuroprotection only after ACOG published its recommendations.

T-013

Group B Streptococcus Infection and Elevated Maternal IL-1β Concentration Are Associated with Early Term Birth. Stephen J Fortunato,[1] Geeta Bhat,[2] Kelsey Mitchell,[3] George Saade,[1] Ramkumar Menon.[1] *[1]The Perinatal Research Center, Centennial Women's Hospital; [2]Ob & Gyn, The University of Texas Medical Branch at Galveston; [3]Epidemiology, Emory University.*

OBJECTIVE: Early term births (between 37–39 weeks of gestation) contribute significantly to infant morbidity and mortality; however, the causality of this condition is still unclear. *Group B Streptococcus* (GBS) is a common bacterial infection and is known to be associated with adverse pregnancy outcomes. We hypothesize that GBS colonization diagnosed between 35 – 37 weeks in otherwise normal pregnancies leads to early term birth due to changes in the maternal-fetal inflammatory status.

METHODS: In this case (GBS positive [n=508]) -control study (GBS negative [n=1,148]) of women delivering at term (>37 weeks gestational age), GBS status (vaginal/rectal swab culture) was obtained from medical records of 1,662 vaginal births. In a nested case-control study, maternal and fetal plasma (n=35 each) biomarkers (IL-1β, IL-2, IL-6, IL-8, and TNF-α) were measured. Student's t-tests determined the differences in gestational age between cases and controls. Associations between GBS status and biomarker concentrations that were significant after Mann Whitney test and false discovery rate (FDR) correction were modeled using logistic regression.

RESULTS: Thirty percent (n=508) of the women in our study were positive for GBS. The average gestational age was significantly reduced to 270.4 ± 13.9 days for GBS positive women compared to 274.4 ± 10.3 days for GBS negative women ($p < 0.0001$). Multivariate analysis indicated that the odds of early term birth was increased by 3 fold in GBS positive women (OR 3.28; 95%CI 2.61 - 4.13; $p <0.0001$). Mean maternal plasma IL-1β (38.93 vs. 11.72 ng/ml; $p <0.0001$) and IL-2 were significantly higher in cases compared to controls (7.02 vs. 6.96 ng/ml; $p=0.004$). Only IL-1β was higher in fetal plasma in cases vs. controls (20.33 vs. 8.18 ng/ml; $p = 0.01$). A 10 ng/ml increase in IL-1β concentration is associated with increased risk for GBS infection (OR: 1.534, CI: 1.013 - 1.75; $p=0.004$).

CONCLUSION: Colonization with GBS even when it does not cause neonatal infection may have detrimental effects to the infant through shortened gestational age. IL-1β concentration in maternal plasma appears to be an indicator of GBS status in early term births. Appropriate intervention and monitoring of GBS positive subjects may prolong gestation and may avoid adverse outcome in infants.

T-014

Serial assessments of Neurobehavioral and Cognitive Development Following Prenatal Maternal Azithromycin (AZI) Therapy for *Ureaplasma* Intra-Amniotic Infection (IAI) in Neonatal Rhesus Monkeys. Victoria HJ Roberts,[1] Nicola D Robertson,[2] Cindy E McEvoy,[3] Robert L Schelonka,[3] Peta L Grigsby.[1,4] *[1]Reproductive & Developmental Sciences, Oregon National Primate Research Center, Beaverton, USA; [2]Behavioral Services Unit, Oregon National Primate Research Center, Beaverton, USA; [3]Pediatrics, Oregon Health & Science University, Portland, USA; [4]OB/GYN, Oregon Health & Science University, Portland, USA.*

Objective: The most prevalent underlying cause of preterm labor, and subsequent neonatal sequlae, is IAI caused by *Ureaplasma* spp. We have previously demonstrated that prenatal maternal AZI therapy for *U.parvum* IAI can reduce fetal lung and neurological injury. The objective of this study was to assess the efficacy of *in utero* AZI treatment on post-natal neurodevelopmental outcomes.

Study Design: At 127-128 days gestation (dGA; term 168 days) long-term catheterized rhesus monkeys received intra-amniotic inoculation with *U.parvum* (10^7 CFU/ml; n=2). Following 10 days of *U.parvum* IAI, each monkey received either maternal AZI (25mg/kg/day) or saline placebo for 10 days. Preterm neonates were delivered at 148 and 152dGA and immediately transferred to our non-human primate (NHP) Intensive Care Nursery. Assessments of neurobehavioral, motor and cognitive development including, reflexive behavior, sensorimotor function and motor maturity were performed by a behavioral specialist 4 times/week over a 3-month follow up period utilizing the Primate Neonatal Neurobehavioral Assessment (PNNA).

Results: The neonate exposed to *U.parvum* IAI (21 days) required a more intense resuscitation at delivery and extensive O_2 support (7 days) during two bouts of mild to severe pneumonia (by chest x-ray). During PNNA testing, this infant showed signs of distraction and inconsistency in performance of repetitive test parameters. In contrast, the AZI-treated infant had normal Apgar scores, showed no clinical signs of pneumonia, required minimal O_2 support (45 min) at birth and reached advanced PNNA testing criterion earlier than the untreated infant.

Discussion: This unique NHP model has the ability to bridge the gap between maternal-fetal medicine and neonatology research by enabling follow-up studies pertinent to the long-term safety aspects of interventional treatment strategies targeted at the delay of infection-associated preterm labor and improved perinatal outcome.

Support: Collins Medical Trust, HD055053, RR00163

T-015

Effects of Tachysystole on Fetal Heart Tracings. Cara C Heuser, Sean Esplin, Calla M Holmgren, Alexandra G Eller, Douglas Richards, Stacey Knight, Erick Henry, Marc Jackson. *Department of Maternal Fetal Medicine, Intermountain Healthcare and the University of Utah, Murray, UT, USA.*

Purpose: Describe fetal heart rate changes associated with tachysystole (TS)

Methods: Retrospective cohort study of all term, singleton, labor patients with TS in a single health system over 26-months. TS was defined by ACOG criteria (>5 UC in 10 minutes averaged over 30 minutes). ACOG fetal heart rate (FHR) category was recorded before, during, and after TS events.

Results: There were a total of 9134 tachysystole events in 6248 deliveries among 6213 patients. Prior to the TS event, 6791 (75.9%) FHR were category I, 2157 (24.1%) were category II, and there were no category III. During the TS event, 5246 (57.4%) FHR were category I, 3888 (42.6%) were category II, and there were no category III tracings. After the TS event, 5521 (69.5%) FHR were category I, 2427 (30.5%) were category II, and there was 1 (0.01%) category III. Characteristics of category II tracings included variable decelerations in 3473 (89.3%), minimal or absent variability in 414 (10.7%), late decelerations in 278 (7.2%), tachycardia in 236 (6.1%), and bradycardia or prolonged deceleration in 182 (4.7%). Table 1 shows FHR category changes before, during, and after all TS events.

FHR category change	Pre TS to During TS (n=8948)	During TS to Post TS (n=7949)	PreTS to Post TS (n=7767)
To Better Category	511 (5.7%)	1462 (18.4%)	759 (9.8%)
No Change	6236 (69.7%)	5727 (72.1%)	5566 (71.7%)
To Worse Category	2201 (24.6%)	760 (9.6%)	1442 (18.6%)

During the TS event, 3205 (40.3%) patients had at least one intervention (terbutaline, decrease/stop Pitocin, position change). In patients who had an intervention and a category II/III tracing during TS (n=1336, in 633 (47.4%) the FHR improved and in 703 (52.5%) the FHR category stayed the same. There were a total of 4744 (60%) patients that had no intervention. In patients with a category II/III tracing and no intervention(n=1795), 829 (46%) improved, 965 (54%) stayed the same, and 1 worsened. There was no difference in the rate of FHR improvement between intervention and non-intervention groups (p=0.54).

Conclusions: TS results in an increased number of FHR category II/III tracings during and after the event. A substantial percent of TS events result in an unfavorable change in FHR category. Variable decelerations occur in almost 90% of category II tracings during TS. FHR improved after TS about half the time even without intervention.

T-016

Predictors of Delivery Mode with Prolonged Labor Induction. Louise Highley, Chad Grotegut, Rebecca Previs, Sarah Dotters-Katz, Leo Brancazio. *Obstetrics and Gynecology, Duke University, Durham, NC, USA.*

Objective: The purpose of this study was to determine characteristics associated with vaginal versus cesarean delivery among women with labor induction lasting over 24 hours.

Methods: Women with live, singleton pregnancies without prior cesarean delivery undergoing a labor induction lasting ≥ 24 hours between September 2006 and March 2009 at Duke University Hospital were identified from a delivery database. Collected variables included demographic data, BMI, parity, induction method, epidural use, oxytocin use, labor duration, infant birthweight, and magnesium use. These were compared by mode of delivery using a multivariate logistic regression model for the outcome cesarean delivery.

Results: 303 women met inclusion criteria. For these women the cesarean delivery rate was 57% (n=172). Relative to women with vaginal deliveries, nulliparous women undergoing cesarean were more likely to be obese (adjusted OR 2.18; 95% CI 1.09, 4.45), have epidural analgesia for a larger proportion of their labor (adjusted OR 1.22; 95% CI 1.04,1.42), and spend more time on oxytocin (adjusted OR 1.06; 95% CI 1.01, 1.11). Delivery by cesarean was less likely among women with a higher initial simplified Bishop score (adjusted OR 0.71 95% CI 0.56, 0.90). For parous women, cesarean delivery was associated with greater duration of both epidural analgesia (adjusted OR 1.52; 95% CI 1.11, 2.26) and oxytocin infusion (adjusted OR 1.33; 95% CI 1.14,1.62). A higher simplified Bishop score was associated with a decreased risk of cesarean delivery for parous women (adjusted OR 0.37; 95% CI 0.14, 0.75).

Conclusions: For nulliparous women undergoing a prolonged labor induction, obesity doubles their risk of cesarean section, whereas for all women, a favorable Bishop score decreases their risk by 30% to 65%. This information may prove useful for clinicians caring for patients with inductions lasting greater than 24 hours.

T-017

Are Serial Ultrasound Measurements Following Cerclage Predictive of Preterm Birth? Laura Houston, Jeffrey Korte, Charles Rittenberg, Roger Newman, Scott Sullivan. *Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA.*

Background: While it is common to follow patients with serial ultrasound measurements following cerclage placement, the appropriate frequency and predictive value of those measurements remains unclear.

Objective: To determine if post-cerclage cervical ultrasound measurements are predictive of preterm birth

Study design: This is an IRB-approved, retrospective observational study of 138 women who underwent history (n=83) or ultrasound indicated (n=55) cerclage placement from September 2006 to July 2011. Serial ultrasound measurements included total cervical length, as well as lengths proximal and distal to the cerclage. Logistic regression was performed to compare these measurements to delivery <35 weeks gestation.

Results: Mean gestational age at delivery for both groups was 35 weeks. Shorter minimum total, proximal, and distal cervical lengths were associated with delivery <35 weeks ($p<.001$). When including both proximal and distal cervical lengths in the model, both remained significant predictors ($p\leq.002$). When controlling for total cervical length, however, the distal measurement was no longer predictive ($p=.63$). Women whose proximal cervical length

decreased to <10 mm had 9.4 times higher odds of preterm delivery, compared to those who remained at least 10 mm (CI 2.7-32.6). When looking at rate of change across measurements, a greater overall decrease in the total cervical length was predictive (p=.001), but not the maximum rate of change observed between any two consecutive visits (p=.07). A continuous variable was created in order to assess whether higher placement of the cerclage improves pregnancy duration. This variable, the proportion of cervix included in a cerclage (distal/total length), was not significantly associated with delivery <35 weeks (p=0.21).
Conclusion: This study provides evidence that total and proximal cervical lengths following cerclage placement provide information regarding risk of preterm delivery. There appears to be more predictive information from the proximal and total cervical lengths, compared to the distal length. Because overall, but not maximum, cervical change was predictive, these findings suggest that fewer ultrasounds may provide as much clinical information as serial ultrasounds.

T-018

Relation of Pre-Pregnancy BMI to Pregnancy Outcome in Women Treated with 17 Alpha-Hydroxyprogesterone Caproate (17P). Sherrine A Ibrahim,[1] Erinn M Hade,[2] Courtney D Lynch,[1] Hetty Walker,[1] Jay D Iams.[1] *[1]Division of Maternal Fetal Medicine, The Ohio State University, Columbus, OH, USA; [2]Center for Biostatistics, The Ohio State University, Columbus, OH, USA.*

Objective

To assess the relationship of maternal pre-pregnancy body mass index (BMI) to rates of recurrent preterm delivery (PTD) in women treated with 17 alpha-hydroxyprogesterone caproate (17P).

Study Design

We conducted a retrospective cohort study of women at high risk for PTD followed in a prematurity prevention clinic from 2005-2010. Women with a current singleton pregnancy who had ≥ 1 prior PTD and a cervical length (CL) < 25 mm < 24 weeks gestation and who were treated with weekly injections of 17P 250 mg IM beginning 16 to 20 weeks' were included. We sought to determine the relationship between maternal pre-pregnancy BMI (underweight+normal vs. overweight+obese) and recurrent PTD. Primary study outcomes were rates of recurrent PTD at < 37, < 32, and < 28 weeks'. Multivariable logistic regression models were used to analyze these associations while adjusting for confounders.

Results

Delivery outcomes for 129 women who met the above criteria were analyzed. 57% were African American; 39% smoked cigarettes. 69 (53%) were treated with cerclage for cervical length < 25 mm despite 17P treatment. 58 women (45%) delivered < 37 weeks'. Compared to underweight+normal weight women, preterm birth in overweight+obese women was more common before 28 weeks' (AOR=3.5, 95% CI: 1.1-11.6) and before 32 weeks' (AOR=2.6, 95% CI: 1.0-6.8). There was no difference between underweight+normal and overweight+obese women in births before 37 weeks' (AOR=1.1, 95% CI: 0.5-2.4).

Conclusions

In contrast to previous studies suggesting that increased BMI is related to a reduced risk of PTD, increased pre-pregnancy BMI in this population of women with cervical length < 25 mm who were treated with 17P was associated with increased rates of recurrent PTD. This observation is unexplained and deserves further investigation.

T-019

Genome Wide Gene-Environment Interaction Analysis of Probiotic Intake in Spontaneous Preterm Delivery. Bo Jacobsson,[1] Oivind Skare,[2] Hakon Gjessing,[2] Anne-Liese Brantsaeter,[3] Verena Sengpiel,[1] Solveig Myking,[2] Ingrid Helene Garman Ostensen,[2] Helle Margrete Meltzer,[3] Margaretha Haugen,[3] Per Magnus,[2] Ronny Myhre.[2] *[1]Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Gothenburg University, Gothenburg, Sweden; [2]Department of Genes and Environment, Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; [3]Department of Food Safety and Nutrition, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway.*

Background: In a recent study we found a reduced risk of spontaneous preterm delivery (sPTD) associated with intake of probiotics. Probiotics have been found to induce specific patterns of gene expression in human mucosa and may influence pregnancy outcome through reduction of inflammatory levels and regulation of immune responses as well as blood pressure normalization during pregnancy. Therefore we hypothesize that intake of probiotics would demonstrate interaction with genetic variation in sPTD.
Methods: Samples were selected from the Norwegian Mother and Child cohort study including 107 000 pregnancies. Gene - environment interaction analyses were performed on 736 preterm case mothers and 712 control mothers. One exposure was milk-based probiotic intake type I containing Lactobacillus acidophilus LA-5 and Bifidobacterium Bb12 a second exposure was milk-based food type II containing Lactobacillus acidophilus LA-5, Bifidobacterium Bb12 and Lactobacillus rhamnosus LGG as well. An additional level of information was investigated by studying biological functions applying KEGG based pathway analysis.
Results: The most associated KEGG pathways were involved in pyruvate metabolism, fatty acid biosynthesis and infection and detoxification of various types. For any milk-based probiotic intake the most associated genes were NDRG1 (p=6.81E-06) for Milk-based food type I combined with type II, CSMD3 (p=7.33E-06) for milk-based food type I, and AHDC1 (p=4.37E-06) for type II which included Lactobacillus rhamnosus LGG and had strongest association in pyruvate metabolism (p=1.73E-12) and fatty acid biosynthesis (p=6.78E-10) as well as pathways of infection suggesting a predominantly influence of L. rhamnosus LGG on this aspect.
Conclusion: Host-microbe gene environment interaction of probiotics intake is observable in the etiology of sPTD, in particular on a pathway level when involving the probiotic L. rhamnosus LGG.

T-020

Modified Shirodkar Cerclage: Pre- and Post-Operative Associations with Latency and Gestational Age at Delivery. Stephanie Lin,[1] Daniel Skupski,[2] Jonathon Reiss,[2] Gary Eglinton.[2] *[1]Obstetrics and Gynecology, NewYork Presbyterian Hospital Weill Cornell Medical College; [2]Obstetrics and Gynecology, New York Hospital Queens.*

Methods:
Retrospective cohort of Shirodkar cerclage placements at our institution 2000-2010—prophylatic (P)(poor history) or ultrasound indicated (USI)(for cervical length (CL) < 1.5 cm). Cerclage was always attempted to be placed as high as possible on the cervix. Exclusions only occurred when required data was missing. Separate least squares regressions were used for P and USI cerclage.
Results:
Out of 260 patients, 165 had required data. USI cerclage n=91 and P cerclage n=74. The table shows baseline characteristics.

Baseline Characteristics

	Prophylactic Cerclage (N=74)	USI Cerclage (N=91)
CL Postop (cm)	4.1 (.8)	3.3 (.7)
Preop CL (cm)	3.8 (.9)	.9 (.7)
GA at Placement (days)	102 (14)	146 (14)
Prior Second Trimester Loss	60 (81.1%)	41 (45.1%)
Prior Preterm Birth (PTB)	64 (86.5%)	28 (30.8%)
Mullerian Anomaly	5 (6.8%)	1 (1.1%)
GA at Delivery (days)	268 (14)	268 (19)
Latency (days)	166 (18)	122 (24)
Sludge	0 (0%)	40 (44%)

Regression analysis using factors seen in the table showed only GA at placement correlated inversely with latency.

Cerclage Regression Analsysis

Prophylactic Cerclage			USI Cerclage		
	Latency Rsq=.43 p<.0001	Gestational Age (GA) at Delivery Rsq=.12 p=.11		Latency Rsq=.43 p<.0001	GA at Delivery Rsq=.1 p=.37
	Coefficient (Std Error)	Coefficient (Std Error)		Coefficient (Std Error)	Coefficient (Std Error)
Intercept	240(16) p<.0001	236(16) p<.0001	Intercept	271(27) p<.0001	271(27) p<.0001
CL Postop	2.3(2.1) p=.28	.21(2.1) p=.31	CL Postop	4.3(3.0) p=.16	4.3(3.0) p=.16
GA at Placement	-.8(.1) p<.0001	.21(.12) p=.07	CL Preop	4.2(3.3) p=.2	4.3(3.3) p=.2
Prior PTB	7.2(4.3) p=.1	7.2(4.3) p=.09	Membranes Exposed	1.1(2.6) p=.68	1.1(2.6) p=.68
Prior Second Trimester Loss	-6.5(3.7) p=.08	-6.5(3.7) p=.08	GA at Placement	-1.2(.16) p<.0001	-.2(.16) p=.29
Mullerian Anomaly	3.5(3.3) p=.3	3.4(3.3) p=.31	Sludge Prop	.02(2.1) p=.99	.01(2.1) p=.99
			Prior PTB	3.3(3.1) p=.29	3.3 (3.1) p=.29
			Prior Second Trimester Loss	-5.8(3.3) p=.11	-5.3(3.3) p=.11
			Mullerian Anomaly	5.4(10.3) p=.6	5.3(10.3) p=.6

Conclusions:
For Shirodkar cerclage placed as high as possible on the cervix, earlier GA at placement is associated with longer latency before delivery. This technique of cerclage placement allows restitution of cervical length to a normal level and delivery at term, possibly eliminating any correlation between cervical length post-operatively and latency.

T-021

A Pilot Study on the Use of Intrapartum Transperineal Ultrasound To Assess Labor Progress. Tsz Kin Lo, Viola Chan, Vivian Ng, Cherrie Yung, Wai Lam Lau, Wing Cheong Leung. *Department of Obstetrics & Gynecology, Kwong Wah Hospital, Hong Kong, China.*

OBJECTIVE

We aimed to assess labor progress using intrapartum transperineal ultrasound.

METHODS

Prior to digital vaginal assessment of labor progress, trasperineal ultrasound in the mid-sagittal plane was performed using portable ultrasound (Esaote Mylab25) to obtain fetal head-to-perineum distance (HPD, the distance between lower-most part of fetal skull and maternal perineum) and angle of progression (AoP, the angle between maternal symphysis pubis and tangent of fetal skull). These were measured by a consultant obstetrician experienced in transperineal ultrasound and a resident with limited ultrasound experience. They were blinded to each other's ultrasound findings. Subsequent digital vaginal examination to determine clinical fetal head station was performed by the attending obstetrician who was blinded to the ultrasound findings. The study was approved by the local research ethics committee and women's written consent was obtained.

RESULTS

A total of 23 pregnant women with normal singleton term pregnancy had ultrasound assessment of labor progress. All delivered vaginally. AoP ranged from 100 to 200° and HPD 0.87 to 5.70cm. There was good repeatability for AoP and HPD, both for the same and different examiners, although AoP tended to be more reproducible (95%CI of intraclass correlation coefficient for inter-observer variability 0.751-0.994 for AoP vs 0.586-0.990 for HPD). (Table 1)

Table 1. Repeatability of ultrasound parameters

sonographic parameter	variability	mean difference (SD)	Intraclass correlation		
			Intraclass correlation coefficient	95%CI	p
AoP	Intra-observer (Consultant)	1.184° (0.975°)	0.995	0.986-0.998	<0.001
	Intra-observer (Resident)	1.600° (1.713°)	0.990	0.963-0.998	<0.001
	Inter-observer (Consultant vs Resident)	5.667° (3.077°)	0.958	0.751-0.994	0.001
HPD	Intra-observer (Consultant)	0.133cm (0.129cm)	0.992	0.981-0.997	<0.001
	Intra-observer (Resident)	0.055cm (0.046cm)	0.994	0.966-0.999	<0.001
	Inter-observer (Consultant vs Resident)	0.228cm (0.251cm)	0.931	0.586-0.990	0.003

AoP was significantly correlated with HPD (AoP=186.752-13.786xHPD, r2=0.622, p=0.001). Clinical fetal head station correlated better with AoP than with HPD (AoP=129.909+11.663x[station], r2=0.385, p=0.002; HPD=2.517-0.471x[Station], r2=0.257, p=0.014).

CONCLUSION

Intrapartum transperineal ultrasound is potentially a reliable technique for quantitative and objective assessment of labor progress. AoP is likely a better ultrasound parameter than HPD.

T-022

Tocolytic Administration for Women with Spontaneous Preterm Labor and Cervical Dilation <24 Weeks Gestation. T Manuck,[1] K Korgenski,[2] M Jackson,[1] R Silver,[1] H Major,[1] F Porter,[1] M Varner.[1] *[1]Obstetrics & Gynecology, Div. of Maternal-Fetal Medicine, Univ. of Utah & Intermountain Healthcare; [2]Pediatrics, Univ. of Utah & Intermountain Healthcare.*

INTRODUCTION: The objective of this study is to characterize tocolytic use and examine perinatal outcomes among women presenting with spontaneous preterm labor (SPTL) and cervical dilation <24 weeks gestation.

METHODS: Data from 1/2000-6/2011 in a single healthcare system were reviewed. Women with a singleton non-anomalous fetus admitted with SPTL and intact membranes, 20.0-23.9 weeks gestation, and with an initial digital cervical exam ≥ 1 cm dilated and ≥ 50% effaced were included. Those with cervical dilation >7cm at presentation were excluded. Decisions regarding tocolytic use and neonatal resuscitation were made by individual patients and physicians. Tocolytics included magnesium sulfate, indomethacin, and nifedipine, used singly or in combination. Women receiving one or more tocolytics were compared to those who did not receive tocolysis. Data were analyzed by chi^2 and t-test.

RESULTS: 148 women met inclusion criteria. The median dilation was 3.5 cm. 84 (56.8%) received at least one tocolytic; 45 (30.4%) received only one, 28 (18.9%) received two, and 11 (7.4%) received all 3. The most commonly used tocolytic was magnesium (n=54, 36.5%), followed by nifedipine (n=44, 29.7%), and indomethacin (n=36, 24.3%). Demographic/Antenatal and Delivery/Outcome characteristics are shown in the table.

	Received Tocolysis N=84	No Tocolysis N=64	p
Demographic & Antenatal Characteristics			
White/Caucasian (n, %)	72 (85.7)	44 (68.8)	0.01
Mean number of prior live births	1.6	1.5	0.80
Prior PTB (n, %)	47 (56.0)	40 (46.9)	0.27
Mean admission gestational age (weeks)	22.7	22.3	0.10
Mean admission cervical dilation (cm)	3.4	3.9	0.10
Received antenatal corticosteroids (n, %)	52 (61.9)	14 (21.9)	<0.001
Delivery & Outcome Characteristics			
Mean admission-to-delivery interval (days)	10.0	4.9	0.02
Pregnant for ≥7 days after admission (n, %)	28 (33.3)	10 (15.6)	0.02
Delivery gestational age (weeks)	24.2	23.2	<0.001
Birthweight (grams)	890	661	<0.001
Neonatal death (n, %)	30 (36.6)	35 (62.5)	<0.001
Neonatal IVH (n, %)	20 (23.8)	9 (14.1)	0.14
Neonatal BPD (n, %)	13 (15.5)	4 (6.3)	0.08
Neonatal NEC (n, %)	2 (2.4)	1 (1.6)	0.73
Major neonatal morbidity* (n, %)	52 (61.9)	41 (64.1)	0.79
Neonatal length of stay (days)**	39.4	30.4	0.40
Maternal length of stay (days)	13.4	7.1	0.01

* defined as IVH, NEC, BPD, or death diagnosed prior to discharge

** among 78 babies admitted to the NBICU

CONCLUSIONS: Approximately half of all women who presented with SPTL and cervical dilation at 20-24 weeks received tocolytic therapy. For many patients, not receiving tocolytics was likely a marker for adoption of non-interventionist management, as the rate of antenatal corticosteroid use was low. The group who received at least one tocolytic had similar antenatal characteristics, but experienced a greater admission-to-delivery interval, delivered approximately one week later, and had a neonatal survival rate almost double that of the No Tocolysis group. Despite this, major neonatal morbidities were similar, trading pre- and peri- viable death for morbidity among those receiving tocolysis.

T-023

The Contribution of Genetic Variation within Regulatory Genes to Spontaneous Preterm Birth. Tracy Manuck,[1,2] Sean Esplin,[1,2] Michael Varner,[1,2] Marc Jackson.[1,2] *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA; [2]Maternal-Fetal Medicine, Intermountain Medical Center, Murray, UT, USA.*

Objective: Recently, the PREBIC Consortium identified genetic variants within several regulatory genes, including ubiquitin-like with ring finger domains 2(UHRF2), MORC family CW-type zinc finger 2 (MORC2), and oxysterol binding protein 2 (OSBP2), associated with spontaneous preterm birth (SPTB) among Caucasian women with intact membranes. We sought to examine if genetic variation in these genes could be confirmed among a different Caucasian population.

Methods: Self-reported Caucasian/White non-Hispanic women with ≥1 early SPTB <34.0 weeks gestation were enrolled prospectively. Women with only term deliveries (37-42 weeks gestation) were recruited as controls. DNA was extracted and genotyped for tag SNPs in the UHRF2, MORC2, and OSBP2 genes using publicly available online gene/SNP databases and a custom Illumina ® genotyping assay. Women or SNPs with ≥10% missing values were excluded. Allelic and genotypic (additive model) association analyses were performed, stratifying by total number of deliveries using PLINK v1.07. The false discovery rate (FDR) adjustment was used to correct for multiple testing.

Results: 109 preterm cases and 116 term controls met inclusion criteria. Cases had an average of 1.8 +/- 1.0 (mean +/- SD) preterm deliveries, with the earliest prior SPTB occurring at 29.2 +/- 3.7 weeks gestation. Controls had 2.1 +/- 1.2 term deliveries. 52 SNPs passed quality filters and were analyzed. None of the SNPs studied were associated with SPTB.

Conclusions: In contrast to the findings presented by the PREBIC consortium, in this high-risk cohort of Caucasian women with at least one SPTB <34.0 weeks gestation, variation in UHRF2, MORC2, and OSBP2 genes was not found to be associated with SPTB.

T-024

Tocolysis for Women with Spontaneous Preterm Labor and Advanced Cervical Dilation. T Manuck,[1] K Korgenski,[2] M Jackson,[1] R Silver,[1] H Major,[1] F Porter,[1] M Varner.[1] *[1]Obstetrics & Gynecology, Univ. of Utah & Intermountain Healthcare; [2]Pediatrics, Univ. of Utah & Intermountain Healthcare.*

OBJECTIVE: Tocolytic regimens are of uncertain efficacy in the setting of advanced cervical dilation (ACD). The aim of this study is to characterize tocolytic use and examine perinatal outcomes among women presenting very

preterm (<32.0 weeks) with spontaneous preterm labor (SPTL) and ACD ≥4 cm.
METHODS: Data from 1/2000-6/2011 in a single large healthcare system were reviewed. Women with a singleton non-anomalous fetus who were admitted with SPTL and intact membranes between 23.0-32.0 weeks gestation and with an initial digital cervical dilation ≥4 cm were included. Women with cervical dilation >7 cm were excluded due to imminent delivery. Women with hypertensive disorders were also excluded. Decisions regarding tocolytics and labor management were made by individual physicians. Tocolytics included magnesium sulfate, indomethacin, and nifedipine, used singly or in combination. Women receiving one or more tocolytics were compared to those who did not receive tocolysis. Data were analyzed by chi2 and t-test.
RESULTS: 383 women met inclusion criteria; 270 (70.5%) received at least one tocolytic; 153 (40.0%) received only one, 96 (25.1%) received two, and 21 (5.5%) received all three. The most commonly used tocolytic was magnesium (n=175, 45.7%), followed by nifedipine (n=126, 32.9%) and indomethacin (n=107, 27.9%). Antenatal and delivery/outcome characteristics are listed in the table. Women who did not receive tocolysis tended to have higher parity and were approximately 0.5 cm more dilated at presentation. Initial perinatal outcomes were similar and did not appear to vary by tocolytic administration.

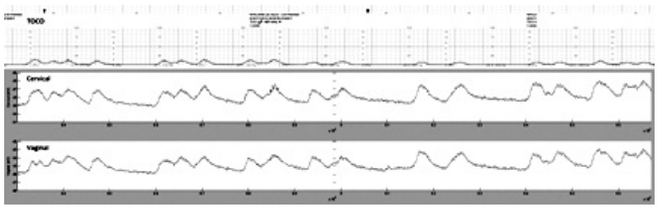
	Tocolysis N=270	No Tocolysis N=113	p-value
Antenatal characteristics			
Mean number of prior live births	1.3	1.8	0.003
Prior PTB (n, %)	165 (61.1)	57 (50.4)	0.05
Mean admission gestational age (weeks)	28.6	29.0	0.13
Mean admission cervical dilation (cm)	5.0	5.4	<0.001
Delivery & outcome characteristics			
Mean admission-to-delivery interval (days)	2.4	2.3	0.78
Pregnant for ≥7 days after admission (n, %)	26 (9.3)	16 (14.2)	0.20
Delivery gestational age (weeks)	28.9	29.3	0.14
Birthweight (grams)	1408	1473	0.26
Neonatal death (n, %)	9 (3.3)	6 (5.3)	0.36
Neonatal IVH (n, %)	58 (21.5)	21 (18.6)	0.52
Neonatal BPD (n, %)	60 (22.2)	20 (17.7)	0.32
Neonatal NEC (n, %)	15 (5.6)	10 (8.9)	0.23
Major neonatal morbidity*	101 (37.4)	37 (32.7)	0.39
Neonatal length of stay (days)**	41.4	36.7	0.27
Maternal length of stay (days)	5.9	7.6	0.11

* defined as the diagnosis of NEC, BPD, IVH, RDS, or death prior to discharge
**among neonates admitted to the NICU

CONCLUSION: As expected, the majority of women presenting with ACD receive tocolysis, and approximately 30% receive more than one tocolytic medication. However, tocolysis does not delay delivery, and the large majority deliver within one week. Initial neonatal outcomes are similar between groups.

T-025

A Novel Device for the Detection of Uterine Contractions. Abimbola Aina-Mumuney,[1] Karin Hwang,[2] Sung-Jin Sunwoo,[2] Karin Blakemore.[1] [1]*GYN/OB, Div of MFM, Johns Hopkins University;* [2]*Biomedical Engineering, Johns Hopkins University.*
The external tocodynamometer (toco) has limited ability at detecting uterine contractions at early gestational ages (GA) and in the obese population. A monitor with the ability to detect contractions in these two clinical settings would be an invaluable clinical tool to add to our armamentarium in the practice of obstetrics.
Objective: To design and test a device that avoids the limitations associated with maternal abdominal contraction monitoring systems, detecting electrical activity from the utero-cervico-vaginal interface.
Methods: A biocompatible elastic ring with extended wings was created with embedded electrodes along the inner ring (cervical) and protruding wing surfaces (vaginal) to detect electrical uterine activity from the vaginal fornix and cervical walls. The device was then placed on the cervixes of late gestation pregnant ewes receiving intravenous oxytocin. The resultant uterine contractions were simultaneously recorded by our test device and toco in 3 sheep. The contraction tracings were then compared to assess the correlation between our test device and the toco by calculating the Spearman correlation coefficient.
Results: A sample tracing of our test device compared to toco is shown in Figure 1. Pooling the results of all 3 experiments, r=0.97, (p<0.0001).



Conclusion: In a late term ovine model, our device is comparable to the toco in terms of contraction detection. It also confirms that the toco is effective in late ovine gestation. Further studies are underway to test the device in the midtrimester to determine if our device will be superior to the external toco, since it bypasses the maternal abdomen. Our device and the toco are being compared to the intrauterine pressure catheter to calculate the sensitivity/specificity of each device at varying GA. This device has the promise to diagnose preterm labor at GA that currently evade preterm labor detection and constitute a time of high fetal wastage. It may also be more effective in the obese patient at any GA. These attributes would have a major clinical impact on the cost and morbidity of preterm labor.

T-026

Are Adverse Perinatal Outcomes in Twin Pregnancies Increased with Maternal Obesity? Sean C Blackwell. *Maternal Fetal Medicine Units Network, Eunice Kennedy Shriver NICHD, Bethesda, MD, USA.*
Objective: To determine whether maternal obesity is associated with increased risk for adverse perinatal outcomes in women with twin pregnancies.
Methods: We studied women in a multi-center placebo controlled trial of 17-OHPC in twin pregnancies in which we found no differences in perinatal outcomes. Those with maternal diabetes (DM), hypertension, asthma, or other medical condition requiring medications were excluded. Maternal obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2. Studied adverse outcomes were; pregnancy loss < 20 weeks, preterm birth (PTB), spontaneous (SPTB) and medically-indicated (MPTB) before 35 weeks' gestation, preeclampsia (PET) or gestational hypertension (GHTN), gestational DM, small for gestational age (SGA: birth weight < 10th %ile), and large for gestational age (LGA: birth weight > 90th %ile).
Results: There were 640 women in the RCT and 27.8% were obese. There were no differences in the rates of GDM, abnormal fetal growth, or PTB between obese and non-obese women (see Table). However the occurrence of hypertensive disorders was more common with maternal obesity.

Outcomes	Maternal BMI < 30 kg/m^2 N=462	Maternal BMI ≥ 30 kg/ m^2 N=178	P
Pregnancy loss < 20 wks	5.19%	7.3%	0.31
GDM	6.5%	10.7%	0.08
PET/GHTN	16.7%	23.6%	0.04
Any PTB	68%	74.7%	0.10
< 28 wks	6.5%	7.9%	0.54
< 32 wks	14.5%	16.9%	0.46
< 35 wks	37.9%	41.6%	0.39
MPTB < 35 wks	11.3%	9.6%	0.53
SPTB < 35 wks	26.5%	32.0%	0.16
SGA	14.7%	12.6%	0.50
LGA	10.3%	14.4%	0.15

Conclusion: The frequency of preeclampsia/GHTN was higher in women with obesity. In our population, maternal obesity was not associated with an increase in perinatal morbidity.

T-027

Cesarean Section Skin Incision in the Extremely Obese Patient: Pfannensteil or Supraumbilical? Brian E Brocato,[1] Jim Y Wan,[2] Luis Gomez,[1] Giancarlo Mari.[1] [1]*Obstetrics and Gynecology, University of Tennessee Health Science Center;* [2]*Preventative Medicine, University of Tennessee Health Science Center.*
Approximately 1/3 of US women of childbearing age are obese. Cesarean delivery is often challenging in this population. There is a paucity of data on the best approach to skin incision in these patients. We hypothesize that no difference exists in wound complication among extremely obese patients who have a Pfannesteil (Pf) or supraumbilical (Su) skin incision. This study compares the outcomes of extremely obese women who have a Su or Pf skin incision.

Design
133 obese patients with a BMI > 40 were selected for this study; 43 had a SU, 90 patients had a Pf . The primary outcome was wound complication including infection, dehiscence or return to the operating room for delayed wound complication. Secondary outcomes and demographics are listed in Table 1. Two-sampled t-test, chi-square test and multivariable linear and logistic regression were performed for statistical analysis.
Results
A Su incision is more likely to result in a wound complication, a classical hysterotomy, EBL >1L and longer operative times (Table 1). After controlling for confounding, there is no difference in wound complication between Su and Pf (Table 2).
Conclusion
A SU is more likely to result in a classical uterine incision, increasing the risk for future pregnancies. Operative time and blood loss are also increased with this approach. There is no difference in wound complications after controlling for confounding factors. We recommend a Pf incision when technically feasible in patients with a BMI greater than 40.

Table 1. Characteristics of the study population

	Su	Pf	p-value
Wound complication	21%	8%	0.029
Classical hysterotomy	67%	8%	<]0.001
EBL > 1L	33%	12%	0.005
LOS > 3 days	77%	66%	0.191
Blood transfusion	16%	6%	0.060
NICU admission	28%	14%	0.063
Operative time	97 minutes	68 minutes	<0.001
Age	32±5	26±6	0.001
Gestational age @ delivery	36.5±2.9	38.3±3.0	0.002
BMI	64±10	56±6	<0.001

Race, hypertension, preeclampsia, tobacco use, previous cesarean section, and number of previous cesarean sections did not differ significantly between the groups

Table 2.Results of multivariate regression analysis

	Odd ratio (95% CI)	p-value
Wound complication	3.7 (0.96-14.3)	0.058
Classical hysterotomy	0.07 (0.02-0.21)	<0.001
EBL > 1L	3.5 (1.3-9.4)	0.012
Total minutes of surgery	regression slope=28.5	<0.001

Variables controlled for: 1. Age, BMI, DM, Smoking, GBS 2. BMI, GA@del 3. and 4. BMI

T-028

Cost-Benefit of Misoprostol Vaginal Insert 100 mcg or 200 mcg Versus Dinoprostone 10 mg for Labor Induction. Judith H Chung,[1] Aaron B Caughey,[2] Barbara Powers,[3] Deborah A Wing.[1] *[1]Obstetrics & Gynecology, University of California, Irvine, Orange, CA, USA; [2]Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR, USA; [3]Clinical Development, Cytokine Pharmasciences, Inc., King of Prussia, PA, USA.*
Objective: To determine the cost-benefit of Misoprostol Vaginal Insert 100 mcg (MVI 100) or 200 mcg (MVI 200) compared to dinoprostone 10 mg vaginal insert.
Methods: Labor characteristics and cesarean delivery rates were obtained from Phase II trials where MVI 100 was compared to dinoprostone, and MVI 100 was compared to MVI 200. Economic data were obtained from review of the literature. Costs were adjusted, as necessary, to 2011 United States dollars (USD) using the medical care component of the consumer price index (CPI). Costs of nursing, unit, and route of delivery were included in the economic analysis.
Results: Time to delivery in minutes was shortest for women receiving MVI 200 (1181 min (1035-1443)), while time to delivery for MVI 100 was 1596 min (11469-1739) and 1650 min (1509-1824) for dinoprostone. MVI 200 recipients also had the lowest cesarean delivery rate (22.9%) as compared to MVI 100 (27.8%) and dinoprostone (26.4%). However, MVI 200 had the highest need for tocolytic administration in labor (9.9%), with tocolytic rates being 5.7% for MVI 100 and 6.2% for dinoprostone. Labor induction with MVI 200 was associated with $809.81 and $835.32 cost savings per individual when compared to MVI 100 and dinoprostone vaginal insert, respectively.
Conclusions: Despite the higher need for tocolytic administration during labor, MVI 200 for labor induction is associated with a significant cost savings as compared to MVI 100 and dinoprostone. This cost-savings is driven by the shorter induction time and lower cesarean delivery rate with MVI 200. This cost analysis may be important information given the rising induction rate and increased emphasis on medical expenditures.

T-029

Effects on Neonatal Biometry Based on Gestational Age at Administration of Betamethasone. Christine K Farinelli,[1] Elysia P Davis,[2] Kim C Winovitch,[1] Christine W Preslicka,[3] Deborah A Wing.[1] *[1]Obstetrics & Gynecology, University of California, Irvine, USA; [2]Psychiatry & Human Behavior, University of California, Irvine, USA; [3]MemorialCare Center for Women, Long Beach Memorial Medical Center, USA.*
Objective: To evaluate when the effect of betamethasone on a neonate's biometry (head circumference, length, birth weight) occurs based on the neonate's gestational age at administration.
Study Design: This is a cohort study of women with singleton pregnancies who received a course of betamethasone prior to 33 weeks gestation. Women with chronic diseases, substance abuse, intrauterine growth retardation, or fetal anomalies were excluded. In order to standardize the neonatal biometry of the subjects who delivered at various gestational ages, neonatal percentiles for each measurement at the time of birth were calculated using Fenton growth curves, which are based on gestational age at birth. The means of the percentiles for the neonatal biometry were compared between subjects who delivered within two weeks of receiving betamethasone and those who delivered after two weeks using Student t-tests.
Results: Over a period of eight years, 297 infants received a single course of betamethasone prior to 33 weeks gestation. 167 subjects delivered within two weeks of receiving betamethasone; 130 subjects delivered after two weeks. Demographics including fetal gender were similar between the groups. The mean of the percentiles for head circumference and birth weight did not differ significantly between the groups (47.1% + 27.4% vs 49.2% + 28.2%, p=0.5; 49.5% + 25.1% vs 55.8% + 27.3%, p=0.05). The mean of the percentiles for length did, however, differ between the subjects who delivered within two weeks (52.9% + 31.0%) and those who delivered two weeks after receiving their course of betamethasone (64.9% + 29.0%, p<0.01). Birth length in the subjects who delivered within two weeks after receiving betamethasone was 12.0% (95% CI 5.1 to 19.0) less than the neonates who delivered after two weeks.
Conclusion: Our results suggest that the gestational age at administration of betamethasone does not affect the neonatal biometric measurements of head circumference and birth weight, but does influence the neonate's birth length. This effect appears to be seen only within the first two weeks after the administration of the betamethasone. Beta-error may account for the lack of association with head circumference and birth weight.

T-030

Effect of Betamethasone on Neonatal Biometry by Gestational Age Percentiles. Christine K Farinelli,[1] Elysia P Davis,[2] Kim C Winovitch,[1] Christine W Preslicka,[3] Deborah A Wing.[1] *[1]Obstetrics & Gynecology, University of California, Irvine, USA; [2]Psychiatry & Human Behavior, University of California, Irvine, USA; [3]MemorialCare Center for Women, Long Beach Memorial Medical Center, USA.*
Objective: To assess the effect of betamethasone on a neonate's biometric (head circumference, length, birth weight) percentiles based on gestational age at delivery.
Study Design: We performed a case control study of women with singleton pregnancies who received a course of betamethasone prior to delivery in a community-based academically affiliated institution. Exclusions for subjects in the study included chronic disease, substance abuse, multiple gestations, intrauterine growth retardation, and fetal anomalies. Controls were similar in gestational age and gender to the cases and delivered within a year of the case. In order to assess the neonatal biometry of the cases versus the controls, Fenton growth curves based on gestational age at birth were utilized to determine the neonate's percentile for each measurement at the time of birth. The means of the percentiles for neonatal biometry were compared using the Student t-test.
Results: 297 infants received a single course of betamethasone prior to 33 weeks gestation. The demographic characteristics with the exception of maternal age did not differ between the groups. The average gestational age of the cases at delivery was 32.4 + 4.0 weeks and that of the controls was 35.8 + 3.1 weeks (p<0.01). We found no differences between the cases and controls with regards to the mean of the head circumference percentiles (47.8% + 27.7% vs 48.1% + 29.2%, p=0.9) or birth weight percentiles (51.8% + 26.2% vs 53.1% + 25.8%, p=0.5). However, mean percentiles for length were significantly different, with that of the cases measuring approximately 9.3% (95% CI 4.5 to 14.0; p<0.01) less than that of the controls (57.9% + 30.7% vs 67.2% + 28.2%).
Conclusion: By standardizing the neonatal measurements using percentiles derived from growth curves based on the gestational age at delivery, we accounted for the difference in gestational age at delivery between cases and controls. Although this is a small study and retrospective in nature, our

analysis nevertheless suggests that betamethasone does not appear to affect the neonate's head circumference or birth weight, but may have an adverse effect on the infant's length at birth when administered for common indications at a community hospital.

T-031

Maternal Obesity Increases the Expression of Components of the Renin Angiotensin System (RAS) in Omental Adipose Tissue. Jie Zhang,[1] Guillermo J Valenzuela,[2] Kristin L Searing,[2] Angela G Massmann,[1] Jorge P Figueroa.[1] *[1]Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, USA; [2]Obstetrics and Gynecology, Arrowhead Regional Medical Center, Colton, CA, USA.*

Obesity in pregnancy is associated with an increased risk for cardiovascular morbidity, in particular hypertension and adverse metabolic outcomes in mother and fetus. Alterations in adipose tissue (AT) function contribute to metabolic dysfunction and are thought to be mediated in part by inflammation. Activation of adipose tissue RAS is thought to play a role in the association between obesity and hypertension.

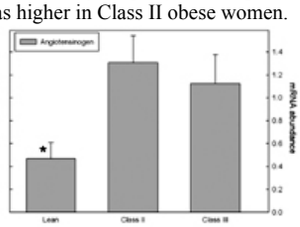
OBJECTIVE: The aim of this study was to determine if components of the RAS are elevated in AT of women with pregnancies complicated by maternal obesity.

METHODS: Subcutaneous and omental AT was obtained at the time of elective C-section after maternal consent under an IRB approved protocol. We studied three groups (Lean: n= 6;Class II: n=9 and Class III: n=9). AT was rinsed in sterile saline and flash frozen. Expression levels of ACE1, ACE2, AT1, AT2 and angiotensinogen (ATG) mRNA were measured by TaqMan real-time PCR using commercial probes for human sequences. RNA was extracted using Trizol reagent, purified and cDNAs prepared. Data are presented as Mean±SEM. Statistical differences were assessed using One way ANOVA.

RESULTS: Women were of comparable gestational age (GA) and by designed stratified by BMI. Ethnic distribution was: Hispanic 13, Caucasian 9 and one each Asian and African-American. Table 1 shows the demographic and antropometric characteristics. Of all the components of the RAS studied only ATG was significantly elevated by maternal obesity (Figure). Although not statistically significant, blood pressure was higher in Class II obese women.

	GA	BMI	Mat wt	Δwt	Birth wt
Lean	39.4	23.8	52.0	12.3	3.1
	±0.23	±0.37	±3.7	±1.32	±0.17
Class II	39.6	38.0	82.0	20.0	3.9
	±0.32	±0.56	±6.3	±3.43	±0.24
Class III	39.3	47.1	105.0	13.9	3.5
	±0.18	±3.00	±8.5	±3.02	±0.22
		*	*		*

GA;weeks;wt:Kg;*p<0.05 by ANOVA



CONCLUSION: Our data show that maternal obesity increases AGT expression in visceral fat of Class II patients. Interestingly, the elevated AGT was observed in Class II obese patients who were the ones who gain the most weight (Δwt) during pregnancy. Thus in agreeement with the clinical findings of an increased incidence of hypertensive disease in obese pregnant women.

Funding: ARMC,HL89840

T-032

Vitamin D Deficiency in Early Pregnancy. Shannon K Flood Nichols, Deborah Tinnemore, Peter G Napolitano, Danille L Ippolito. *Department of Obstetrics and Gynecology, Madigan Army Medical Center, Tacoma, WA, USA.*

Objective: Vitamin D deficiency is a common problem in reproductive age women in the United States. The effect of vitamin D deficiency in pregnancy is unknown, but has been associated with a variety of adverse pregnancy outcomes including preeclampsia, growth restriction, and preterm delivery. The objective of this study was to analyze the relationship between vitamin D deficiency in the first trimester and subsequent adverse pregnancy outcomes.

Methods: This is a retrospective cohort study. Plasma was collected in the first trimester from 310 primigravid women with singleton gestations without significant medical problems. Competitive enzymatic vitamin D assays were performed on banked plasma specimens and pregnancy outcomes were collected after delivery. Researchers were blinded to the pregnancy outcomes while performing the vitamin D assays.

Results: Vitamin D concentrations were obtained from 108 patients (mean age 23.6 years, range 19-37 years). Seventy-one percent of our study population was vitamin D deficient with a serum concentration less than 30 ng/mL (mean serum concentration 27.8 ng/mL, range 13.8-71.6 ng/mL). Logistic regression was performed adjusting for age, race, body mass index, tobacco use, and time of year. Adverse pregnancy outcomes included preeclampsia, growth restriction, preterm delivery, gestational diabetes, and fetal loss. There was no association between vitamin D deficiency and adverse pregnancy outcomes with an adjusted odds ratio of 1.09 (*p* value 0.851).

Conclusion: Vitamin D deficiency does not correlate with adverse pregnancy outcomes in this study population. However, the high percentage of affected individuals highlights the prevalence of vitamin D deficiency in young, reproductive age women.

T-033

Outcomes across a 10-Year Period in Placenta Accreta-Related Pathologies. V Gupta,[1] A Singavi,[1,2] S Zamudio,[1] M Pawlic,[1] R Levat,[1] M Alvarez,[1] A Al Khan.[1] *[1]Ob/Gyn, Hackensack University Medical Center, Hackensack, NJ, USA; [2]St. Georges Univ. School of Medicine, Grenada.*

The incidence of placenta accreta [pA], increta [pI] and percreta [pP] has risen from 1/30,000 to 1/2500 pregnancies, >10-fold since the 1950s. Prior cesarean delivery combined with placenta praevia confers the greatest risk: 67% of women with 3 prior cesareans and a praevia will develop an pA, pI or pP. Particularly understudied is the excess vascularity that develops in the lower uterine segment and is the underlying cause of hemorrhage. Hackensack University Medical Center's Center for Abnormal Placentation (CAP) is a regional referral center for pAIP. This study was conducted to 1) assess outcomes pre- vs. post adoption of a multi-disciplinary team for management of pAIP, and 2) test whether clinically assessed vascularity correlates with the depth of uterine invasion. **Methods**: We reviewed all records since 2000 with a pathologically confirmed diagnosis of pAIP. Maternal/neonatal charts were abstracted for demographic data, surgical procedures and outcomes. The 62 cases were divided into an early cohort (2000-2006, 8 A, 8I, 8P), before care was standardized vs. a late cohort (2007-2011 14 A, 13 I, 11 P), after team management was instituted. Intra-operative photos and/or documentation in the surgical report of excess vascularity was required. Data were analyzed using ANOVA, chi square or the Kruskal-Wallis test. **Results**: Early vs. late cohorts did not differ in demographics or risk factors. All babies were AGA. The presence of both praevia and prior Cesarean was greater in I (80%) and P (100%) than in A (18%, p<0.05). Excess vascularity was greater in I ( ) and pP () than A (23%, P<0.05). Estimated blood loss in the later cohort decreased by 65% in accreta (p<0.01), 35% in increta (NS) and 49% in percreta (P<0.05). Units of packed RBCs administered decreased in A and P (55%, p<0.05) but not I (8%). SICU admissions decreased (46% vs. 21% p<0.05) in the later cohort, but NICU admissions/length of stay (LOS) increased due to earlier delivery. Maternal LOS did not differ significantly, but increased in variability, due to a greater number of staged surgeries in the later cohort. **Conclusions**: Management in the later cohort improved outcomes in terms of blood loss and administration of blood products. However LOS and NICU admissions increased. Needed are additional data on the degree to which these pathologies progress, so that delivery time is optimized.

T-034

Adverse Pregnancy Outcomes and Risk of Subsequent Rheumatoid Arthritis. Kimberly K Ma,[1] Katherine A Guthrie,[2] J Lee Nelson,[2,3] Carin E Dugowson,[3] Hilary S Gammill.[1,2] *[1]Ob/Gyn, Univ. of Washington; [2]Dept. of Clinical Research, Fred Hutchinson Cancer Research Center; [3]Rheumatology, Univ. of Washington, Seattle, WA, USA.*

Objective: Parity is a protective factor for rheumatoid arthritis (RA). However, some adverse reproductive outcomes are associated with an increased risk of subsequent RA compared with uncomplicated pregnancies. We sought to determine whether low birthweight (LBW) and preterm birth (PTB) are associated with subsequent development of RA.

Study Design: We conducted a secondary analysis from a population-based prospective study of newly diagnosed RA cases and age-matched controls. Parous women were included in this analysis. Multiple gestation pregnancies were excluded. The primary outcome measure for logistic regression analysis was disease status (RA vs. control), with exposures of prior PTB and prior delivery of an infant with LBW (<2500 grams), very low birthweight (VLBW, <2000 grams), and extremely low birthweight (ELBW, <1500 grams). Since positivity for rheumatoid factor (RF) is associated with more severe RA, the subgroup of RF-positive RA cases was also considered. Characteristics of cases and controls were compared via t-test and chi-squared test.

Results: 202 RA cases and 1102 control subjects were available for analysis. Cases were more likely to be nonwhite and current/former smokers. Age and gravidity were similar between groups. Prior PTB was more common among RA cases compared with controls, but this was not statistically significant (17% vs. 13%, p=0.14). Birthweight results are shown in Table 1. Prior ELBW was associated with RA, and prior VLBW and ELBW were associated with RF-

positive RA. Prior LBW occurred more often in RA and RF-positive RA cases than controls, but the differences were not statistically significant.

Conclusion: Compared to those with uncomplicated pregnancies, women with a prior VLBW or ELBW delivery had a higher risk of RA, particularly RF-positive RA. This association may reflect common risk factors for pregnancy complications and RA. Alternatively, complicated pregnancy itself may confer risk for later life RA.

Risk of subsequent RA according to pregnancy history

	Controls (n=1102)	RA Cases (n=202)			RF-positive RA cases (n=102)		
	n (%)	n (%)	RR (95% CI)	p value	n (%)	RR (95% CI)	p value
LBW	151 (14)	37 (18)	1.4 (1.0-2.1)	0.09	19 (19)	1.4 (0.9-2.4)	0.17
VLBW	14 (1)	6 (3)	2.4 (0.9-6.3)	0.08	5 (5)	4.0 (1.4-11.4)	0.009
ELBW	6 (1)	4 (2)	3.7 (1.0-13.2)	0.04	3 (3)	5.5 (1.4-22.5)	0.02

T-035

Modeling Amniotic Fluid Volume in Normal Singleton Pregnancies with Quantile Regression. Songthip T Ounpraseuth,[1,2] Adam T Sandlin,[1] Horace J Spencer,[2] Everett F Magann.[1] *[1]Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA; [2]College of Public Health Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.*

Objective: To provide reliable amniotic fluid volume (AFV) for gestational age (GA) centile charts in normal singleton pregnancies using quantile regression (QR).

Methods: Our analysis is based on the AFV of 379 normal singleton pregnancies. AFVs were determined by dye-dilution techniques or by direct measurement at cesarean delivery. AFV centiles were estimated using QR, which provides a flexible semi-parametric approach of estimating rates of change across the entire distribution of AFV rather than just in the mean as with regular linear regression.

Results: The study included 379 women between 16 and 41 weeks gestation of which 131 (35%) were Caucasian and 248 (65%) non-Caucasian. The mean age (± SD) of the mothers was 26 (± 6) years, mean GA at delivery was 34.6 (± 6) weeks and the mean AFV was 789 mL (± 900 mL), 102 women (26.9%) were nulliparous and 277 women (73.1%) were parous (parity range, 1 to 8). Given that the distribution of AFV was skewed, all subsequent analyses were performed on a logarithmic transformation of AFV. To improve on the shortcomings of standard regression models, QR was used to determine the association between AFV and GA. In particular, a second-order QR model indicated a nonlinear relationship with the upper 25% ($\geq 75^{th}$ percentiles, $p \leq 0.05$ for H_0: $\beta_1=\beta_2=0$) of AFV across GA. The expected 5^{th}, 10^{th}, 90^{th} and 95^{th} percentiles of AFV for a given GA are shown in Table 1. The QR equation to predict the 95^{th} percentile of the logarithmic AFV (mL) was $-1.58 + [0.70 \times GA - 0.01 \times GA^2]$.

Amniotic Fluid Volume (AFV) Percentile Values in Relation to Gestational Age by 2nd Order Quantile Regression

	AFV (mL) Percentiles			
Weeks of Gestation	5th	10th	90th	95th
16	134	134	704	694.7
20	129.2	157.9	1455	1986.6
24	129.8	179.8	2297.1	3839.9
28	135.8	198	2770.2	5016.9
32	148.1	210.8	2551.9	4430.5
36	168.2	217	1795.6	2644.7
40	198.9	216	965.1	1067.1

Table 1

Conclusion: This study defines normative centile charts for each gestational week in normal singleton pregnancies using QR. The use of QR relaxes the stringent assumptions of standard regression and overcomes its limitations; thus, allowing for the examination of association between the response variable and predictors beyond the mean for any percentile of the response (e.g., 25^{th}, 50^{th}, 75^{th}, 95^{th}, etc.).

T-036

Can Maternal Hydration Improve the Amniotic Fluid Inndex and the Pregnancy Outcome in Third Trimester Isolated Oligohydramnios? A Proposal of Therapeutic Plan. Tito S Patrelli,[1] Laura Franchi,[2] Giovanni Piantelli,[3] Alberto Bacchi Modena.[4] *[1]Dpt of Obstetrics, Gynecology and Neonatology, University of Parma, Parma, PR, Italy; [2]Dpt of Obstetrics, Gynecology and Neonatology, University of Parma, Parma, PR, Italy; [3]Dpt of Obstetrics, Gynecology and Neonatology, University of Parma, Parma, PR, Italy; [4]Dpt of Obstetrics, Gynecology and Neonatology, University of Parma, Parma, PR, Italy.*

Amniotic fluid is important for the maintenance of foetal well-being, because of its physical, functional and homeostatic functions. So its deficiency, that is oligohydramnios, can have multiple impacts on the good prosecution of the pregnancy. In some cases there are no foetal and/or maternal evident causes, so we have a particular condition, i.e. isolated oligohydramnios.

The aim of our study was the validation of maternal oral hydration therapy as non-invasive therapy for resolution or improvement of isolated oligohydramnios in the third trimester of pregnancy.

We conducted a prospective, randomised, controlled study on 66 pregnancies complicated by idiopathic oligohydramnios in the third trimester (Group A), with a control group of 71 women with physiological pregnancies (Group B). The study group was divided into two subgroups (Subgroup A vs B) according to the volume of oral hydration (1500cc vs 2500 cc). We considered AFI index to compare the effectiveness of the therapy. The difference between the group A and B was highly significant ($p<0.001$); while there was non statistically significant differences between Sub-groups A and B.

Our data show that in pregnancies complicated by isolated oligohydramnios the hydration therapy improves significantly the quantity of amniotic fluid.

T-038

Electronic Fetal Monitoring: Is Variability Alone Enough? Zachary Rubeo, Jaclyn Coletta, Elizabeth Murphy, Cynthia Gyamfi. *Department of Obstetrics & Gynecology, Columbia University Medical Center, New York, NY, USA.*

Objective: According to the 2008 NICHD criteria for fetal heart rate monitoring, the presence of moderate variability is thought to reliably exclude fetal acidemia. Our objective was to determine if the presence of moderate variability alone - without accounting for other features of the fetal heart tracing such as accelerations or decelerations - can characterize a normal fetal arterial pH.

Methods: This is a retrospective case-control study at a single institution of patients with a fetal umbilical arterial pH <7.00 matched to the next consecutive delivery that resulted in an umbilical arterial pH >7.20. The variability of each tracing (absent, minimal, moderate or marked) was assessed by a group of three individuals certified to interpret electronic fetal heart rate monitoring according to NICHD criteria. The fetal heart variability was then compared between pH groups.

Results: Twenty-four cases (pH <7.00) and twenty-four controls (pH >7.20) were identified. There were no tracings with absent or marked variability in either group. When comparing the two groups, there were significantly more tracings with minimal variability in the pH <7.00 group versus the pH >7.20 group (41.7% vs. 12.5% p=0.02) and significantly more tracings with moderate variability in the pH >7.20 group versus the pH <7.00 group (87.5% vs. 58.3%, p=0.02). Among the control group alone, there were significantly more tracings with moderate compared with minimal variability (87.5% vs. 12.5%, p<0.001).

Variability & fetal pH

	Fetal pH <7.00, n=24	Fetal pH >7.20, n=24	p-value
Minimal variability	10 (41.7%)	3 (12.5%)	0.02
Moderate variability	14 (58.3%)	21 (87.5%)	0.02
p-value	0.26	<0.001	

Conclusion: The presence of moderate variability alone can characterize a normal fetal pH, however minimal variability alone failed to identify fetuses with acidemia.

T-039

Impact of the Tohoku Region Pacific Coast Earthquake and Tsunami Disaster on Perinatal Outcome. Junichi Sugawara,[1] Hiroshi Chisaka,[2] Tetsuro Hoshiai,[1] Kazuyo Sato,[1] Tomohisa Ugajin,[3] Shogo Shigeta,[3] Yoshimi Hasegawa,[2] Kunihiro Okamura,[4] Nobuo Yaegashi.[1] *[1]Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai, Miyagi, Japan; [2]Obstetrics and Gynecology, Ishinomaki Red Cross Hospital, Ishinomaki, Miyagi, Japan; [3]Obstetrics and Gynecology, Kesennuma City Hospital, Kesennuma, Miyagi, Japan; [4]Obstetrics and Gynecology, Tohoku Kosai Hospital, Sendai, Miyagi, Japan.*

Background

On March 11, 2011, a catastrophic earthquake measuring 9.0 on the Richter scale, and the associated devastating tsunamis struck the Tohoku region of Japan, leading to over 15,000 deaths and about 4,000 people missing. In the present study, we investigated the short-term perinatal outcomes in a group of women who were pregnant during the earthquake and tsunami disaster, and compared outcomes to those before the disaster.

Methods

We utilized all birth records from five hospitals and six private clinics cited along the Pacific in Miyagi prefecture. Births from March 11, 2010 through August 31, 2010 were categorized as pre-earthquake and those from March 11, 2011 through August 31, 2011 comprised the post-earthquake group.

Results

The numbers of births in the pre- and post-earthquake groups were 2,42 and 1,824, respectively, representing a 10.7% decrease after the earthquake. No significant differences were observed between the pre- and post-earthquake groups for the general maternal index. Interestingly, the ratio of preterm birth post-earthquake (3.9%) was significantly less than the pre-earthquake ratio (5.1%). We also observed higher Apgar scores in the post-earthquake group (9.26±0.61 *vs*. 9.14±0.59). No statistically significant differences were observed in the length of gestation, birth weight, or the ratio of low birth weight neonates. The percentage of women affected by pregnancy-induced hypertensive disorders increased non-significantly after the earthquake (3.58 *vs.* 4.28%).

Conclusion

Despite the severe and widespread trauma resulting from this disaster, there were minimal short-term effects on perinatal outcome.

T-040

Systolic Blood Pressure Predicts Levels of Placental Growth Factor (PlGF) but Not the Soluble Tyrosine Kinase 1 Receptor (sFlt-1) in Healthy Nulliparous Women throughout Pregnancy. Katherine M Johnson, Chloe Zera, Thomas McElrath, Ann Thomas, Louise Wilkins-Haug. *Division of Maternal Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

BACKGROUND: Levels of angiogenic factors vary widely among studies in normal pregnancies. Cross sectional studies have established that certain baseline maternal characteristics are associated with alteration in angiogenic factors. Given that pregnancy requires maternal cardiovascular adaptation including endothelial activation and alteration of the angiogenic milieu, we hypothesize that maternal characteristics associated with endothelial dysfunction explain some variation in the longitudinal pattern of angiogenic factors during normal pregnancy.

METHODS: This study used data collected prospectively from a large, multi-center cohort, with analysis of sFlt-1 and PlGF in women without medical co-morbidities who delivered singletons at term (n=1368). The effect of multiple covariates, including blood pressure over time, on inter-subject variation of sFlt-1 and PlGF was examined in longitudinal multivariate models.

RESULTS: Systolic blood pressure (SBP) throughout pregnancy correlated positively with PlGF (β =0.000687, p=0.0375), but SBP was not significantly associated with sFlt-1 over time. The positive association between SBP and PlGF remained, even when adjusting for African American race, and it was strengthened when accounting for pre-pregnancy body mass index (BMI) (β =0.001135, p =0.0010). BMI >30 had a negative effect on PlGF (β =-0.04795, p<0.0001). African American race had a positive effect on PlGF (β =0.03171, p=0.0002).

CONCLUSIONS: This study is unique in its longitudinal collection of angiogenic data during pregnancy. We observed SBP correlates with inter-subject variation of PlGF, although this was not the case for sFlt-1. This trend in normal pregnancies with respect to PlGF is opposite that observed in pre-eclamptic pregnancies, where higher mean arterial pressure is associated with lower PlGF. This observation has clinical implications for studies of abnormal placentation, since it implies the relationship of blood pressure over pregnancy and PlGF is disrupted in these disorders. Moreover, since pre-pregnancy BMI positively confounds the relationship between SBP over time with PlGF, it is important to account for covariates that modify blood pressure in interpreting the longitudinal variation of PlGF in normal pregnancies.

T-041

Real Time Cerebral Hemodynamics Evalution by Time-Resolved Spectroscopy (TRS-20) during Cesarean Operation. Kaori Yamazaki, Hiroaki Itoh, Keiko Muramatsu, Kotomi Nagahashi, Yuki Nakamura, Naoaki Tamura, Toshiyuki Uchida, Kazuinao Suzuki, Naohiro Kanayama. *Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka-ken, Japan.*

Objective; Cesarean section is usually one of safe operations; however, sever maternal brain damage could occur in rear cases of massive bleeding or sever hypertension during operation. Time-resolved spectroscopy (TRS-20) is the system to measure real time oxyhemoglobin, deoxyhemoglobin, oxygen saturation (%) in alive tissues and organs. We applied TRS-20 to the assessment of brain oxygen saturation during cesarean operation.

Methods; We measured oxygen saturation of anterior portion of the cerebrum in 18 women during cesarean section at Hamamatsu University School of Medicine University Hospital from May 2010 to January 2011. 18 cases were 13 cases with no maternal complications (control group;9 cases of repeat cesarean section, 2 cases of fetal growth restriction, 1 case of twins, 1case of placenta previa), 4 cases complicated with preeclampsia (preeclampsia group), 1 case of massive bleeding due to adhesive placenta previa (total bleeding 3750g).

Result; Average oxygen saturation was 67.2%(before operation)→66.5%(after operation)(control group), 73.6%→72.7%(preeclampsia group). In a case of massive bleeding due to adhesive placenta previa, oxygen saturation rapidly decreased (67.2%→54.2%) concomitant with an increase of bleeding.

Conclusion; It was suggested that Time-resolved spectroscopy (TRS-20) is useful for monitoring maternal brain oxygen saturation during cesarean operation, especially in cases of massive bleeding and preeclapsia.

T-042

Four Developmental (Embryonic) Mullerian Diseases – Mullerianosis. Ronald E Batt,[1] John Yeh.[2] *[1]Gynecology-Obstetrics, State University of New York at Buffalo, Buffalo, NY, USA; [2]Vincent Department of Obstetrics and Gynecology, Masssachusetts General Hospital, Boston, MA, USA.*

INTRODUCTION The theory of mullerianosis predicts that embryonic mullerian tissue - misplaced during organogenesis - results in the formation of four benign mullerian diseases: developmental adenomyosis, endometriosis, endosalpingiosis, and endocervicosis. (Batt et al. Histol Histopathol 2007;22:1161.)

OBJECTIVE To present embryonic and post-embryonic evidence in support of the existence of four benign developmental mullerian diseases.

METHODS We identified four developmental mullerian diseases.

RESULTSDevelopmental adenomyosis has been described in the uterus of a nine-month-old human female fetus and in an infant. (Meyer R. Berlin: S. Karger 1899, 154pp, 11 pl.) "Primitive endometrium…expressing CA125 and oestrogen receptor," consistent with developmental endometriosis, has been identified in four human fetuses. (Signorile et al. J Exp Clin Cancer Res 2009;28:49.) A mullerian choristoma consistent with developmental endosalpingiosis has been described. (Batt et al. Int J Gynecol Pathol 2010;29:546) A mullerian choristoma was reported, consistent with developmental endocervicosis. (Steele et al. Am J Surg Pathol 1982;6:173.)) Also, mullerian choristomas - each composed of endocervical and endometrial and endosalpingeal tissue - have been identified in the mesosalpinx (Lim et al. Int J Gynecol Pathol 2003;22:209.), spinal cord (Barresi et al. Histol Histopathol 2006;21:1111.) and ureter (Li et al. Urology 2007;69:12-08.e9-11.)

DISCUSSION Four developmental mullerian diseases have been identified, two in human fetuses and two in adult females. Developmental adenomyosis and endometriosis have been identified in human fetuses. Mullerian choristomas consistent with developmental (embryonic) endocervicosis and endosalpingiosis have been identified in adult human females. We postulate that developmental (embryonic) mullerian choristomas with one mullerian tissue can be diagnosed in adult females with a high degree of probability when three criteria are met: [1] no evidence of pelvic endometriosis, [2] no direct communication with the endocervix, endometrium, or endosalpinx, and [3] no history of surgery on the reproductive organs.

CONCLUSIONS We postulate that exceptional cases of adenomyosis, endometriosis, endosalpingiosis, and endocervicosis are developmental in origin.

T-043

SLC35C2 Knockout Impair Mouse Reproductive System. Ying Chen, Kai Wang, Richard Leach. *OB/G & Reproductive Biology, Michigan State University, grand rapids, MI, USA.*

Background: Solute carrier family 35, member C2 (SLC35C2) is a membrane transporter located in Golgi membrane. Its major function is GTP-fucose transport. Furthermore, this gene plays important role in cancer cell invasion and hypoxia response. Inhibition SLC35C2 gene expression in human ovarian cancer and trophoblast cells results in diminished cell proliferation and increased cell invasion taken together, these data indicated that SLC35C2 plays important role in cell invasion/mobility in vitro cell model. However, SLC35C2 gene true function remains unknown.

Methods and results: To study its role, we established a SLC35C2 knockout mouse model by crossing male SLC35C2 chimeras with C57Bl/6 female mice resulting in SLC35C2 +/- mice. There is no visible abnormal development or behavior in heterozygous (n=120), and no gender bias (male: female=48%:52%). Litter sizes are significantly smaller (5 pups vs. 10 pups / litter) when compared to controls. To investigate further the decreased litter sizes, IVF was performed with gametes from SLC35C2 +/- mice which revealed fertilization was similar to controls (63% vs. 80%), but the development capacity (from 2-cell to 8-cell) is significant lower than controls (70% vs. 89%, p=0.02). SLC35C2 +/- mated mice resulted in no SLC35C2 -/- pups from a total 115 offspring and we confirmed the arrest to occur prior to E8.5-day of development.

Conclusion: SLC35C2 +/- and -/- mice exhibit subfertility and embryonic lethality respectively. SLC35C2, a fucose transporter, plays an important role in early development. Precisely which unfucosylated protein or proteins that cause these developmental effects is the focus of ongoing investigation.

T-044

The Effect of Maternal Androgenization on Fetal Gluconeogenesis. Fiona Connolly,[1] Katharina E Spath,[1] Kirsten Hogg,[1] Mick T Rae,[2] Alan S McNeilly,[1] W Colin Duncan.[1] *[1]MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom; [2]School of Life Sciences, Edinburgh Napier University, United Kingdom.*

Exposure of a female fetus to excess testosterone *in utero* results in a polycystic ovary syndrome (PCOS)-like phenotype in the adult. PCOS is associated with metabolic dysfunction including non-alcoholic fatty liver disease. We previously found the development of hepatic steatosis along with alterations to hepatic gluconeogenic gene expression in young adults in an ovine model of prenatal androgenization. We hypothesized that alterations which culminate in young adults initiate during fetal life and present as functional differences in metabolically active tissues. An ovine model of midgestation androgenization was used to determine if gluconeogenic gene expression was altered in the fetal liver and kidney. Pregnant ewes treated twice weekly with testosterone propionate (TP;100mgs, n=8) or vehicle control (C, n=6) from day 60 of gestation were sacrificed at day 90 and fetuses collected. Tissue was snap frozen for qRT-PCR and also stored in bouins for immunohistochemistry. Maternal androgenization resulted in decreased renal expression of phosphenolpyruvate carboxykinase (*PEPCK*; $P<0.01$) and a trend towards reduced expression of glucose-6-phosphatase (*G6P*; $P=0.10$), with no difference in hepatic expression of either enzyme after maternal TP. To assess mechanisms in differential tissue regulation steroid receptor location and expression and regulatory pathways for inhibition (insulin) and stimulation (glucocorticoids) were assessed. Co-localization of androgen receptor (AR) and estrogen receptor α (ERα) with PEPCK and G6P occured in the fetal kidney, and there was more *AR* ($P<0.05$) and *ERα* ($P<0.05$) than in the liver. Glucocorticoid receptor (*GR*) expression was similar between tissues. Although *HSD11β2* was expressed in the fetal kidney and not the liver, there was no effect of TP exposure. Though renal or hepatic insulin receptor (*IR*) expression was not changed in response to *in utero* TP excess, renal expression of insulin receptor substrateI (*IRSI*; $P<0.01$) was significantly increased. This is a novel investigation assessing the effect of androgenization on fetal gluconeogenesis and the first to report a significant decrease in renal expression of the major gluconeogenic enzymes resulting from *in utero* testosterone excess, suggesting a possible role in the progression towards adult related disease.

T-045

Developmental Vitamin D_3 Deficiency Disrupts Female Reproductive Physiology. Joseph Davis,[1] Marlena Petti,[1] Thalia Segal,[2] Genevieve Neal-Perry.[1] *[1]OBGYN, Montefiore Med Center and Einstein Medical School, Bronx, NY, USA; [2]OBGYN, North Shore, Manhasset, NY, USA.*

Vitamin D_3 (VD_3) deficiency has reached near epidemic levels, especially in reproductive aged women. Recent studies suggest VD_3 and the VD_3 receptor have important roles in reproduction and metabolism raising the possibility that developmental (*in utero* and perinatal) VD_3 deficiency may adversely affect reproductive physiology and metabolism in at risk offspring. **Objective**: To determine if developmental VD_3 deficiency affects hypothalamic-pituitary-ovarian physiology or glucose tolerance. **Methods**: Female offspring (n=14-24) born to dams fed vitamin D_3 sufficient (VD_3+; controls) or deficient (VD_3-) diets throughout gestation and lactation and then maintained on a VD_3+ diet were used to assess the effect of developmental VD_3- on the pubertal transition (defined by vaginal opening (VO) and 1st estrus), estrous cycling (monitored with vaginal cytology for 4 wks), and glucose tolerance. The effect of developmental VD_3- on glucose tolerance and ovarian follicular development in young adults was determined with glucose tolerance tests and immunohistochemistry, respectively. The effect of developmental VD_3- on E_2 negative feedback and pituitary responsiveness to GnRH peptide was determined in gonadectomized mice primed with E_2 and P. Data is analyzed with 2-way ANOVA or non-parametric tests. **Results**: Developmental VD_3- did not affect the age of VO, 1st estrus or pituitary responsiveness to E_2 negative feedback or GnRH peptide challenges. Mice with developmental VD_3- had better glucose tolerance ($p<0.01$), oligoovulation ($p<0.01$), extended diestrus and estrous cycles ($p<0.01$), more primordial ($p<0.01$) and primary ($p<0.01$) follicles and tended to have more preantral follicles than controls. **Conclusion**: Our data suggest that developmental VD_3- does not disrupt puberty or pituitary responsiveness to E_2 negative feedback or GnRH peptide. Our studies do not rule out the possibility that developmental VD_3- adversely affects E_2 positive feedback or the hypothalamus. Interestingly our data suggest developmental VD_3- affects glucose tolerance; however insulin tolerance tests are planned to better interpret these data. Developmental VD_3- is associated with oligoovulation, extended follicular phases (dietrus), increased numbers of early stage follicles and arrested follicular development. This reproductive phenotype is reminiscent of PCOS.

Support: NIH HD066355, The Zondek Award

T-046

The Homeodomain Transcription Factor LIM1/Lim1 Is Expressed in Developing and Adult Human and Mouse Endometrium. Louie Ye,[1] Jemma Evans,[2] Caroline E Gargett.[1] *[1]The Ritchie Centre, Monash Institute of Medical Research, Melbourne, Victoria, Australia; [2]Prince Henry's Institute, Melbourne, Victoria, Australia.*

Background: Lim1 encodes a homeodomain transcription factor required for head, kidney and female reproductive tract development in the murine embryo. In the developing female reproductive tract Lim1 expression was first detected in the Müllerian ducts (MD). We recently reported the differentiation of human embryonic stem cells (hESC) into MD-like epithelium in vivo using a tissue recombination model.[1]

Objectives: To localise LIM1 expression in the hESC-derived female reproductive tract developmental model, examine LIM1/Lim expression in adult human and mouse endometrium and determine whether Activin A regulates LIM1 expression in human endometrial epithelial cells.

Methods: Tissue recombinants comprising GFP-labelled hESC-derived embryoid bodies and neonatal mouse uterine mesenchyme were incubated for 5 days in vitro and then transplanted under the kidney capsule of immunocompromised NSG mice for 2-8 weeks. Human female reproductive tract epithelium was identified in the grafts as described.[1] Dual colour immunofluorescence and qRT-PCR were used to detect LIM1 expression in female reproductive tract-like epithelium in recombinant tissues, full thickness human and mouse endometrium, and in endometrial cancer cell lines. ECC-1 cells were incubated with 50-150 ng/ml Activin A for 2-24 hrs and LIM1 assessed by qRT-PCR.

Results: In the recombinant human reproductive tract model, the number of $LIM1^+CK18^+$ epithelial cells diminished with time, although there was some persistent expression in mature reproductive tract epithelium. In human endometrium, significantly more $LIM1^+$ cells were observed in luminal (LE) and glandular epithelium (GE) of the functionalis than the basalis ($P<0.05$). Significantly more $LIM1^+$ cells were found in the proliferative and late secretory stages of the menstrual cycle compared to early secretory stage ($p<0.05$). In mouse endometrium, LE and GE expressed Lim1 which was maximal

in proestrus and minimal at estrus. Primary endometrial cancer tissues and Ishikawa, HEC1A and ECC1 cells all expressed LIM1. We are currently examining the effect of Activin A on LIM1 expression in ECC1 cells.
Conclusion: These studies demonstrate previously unreported Lim1/LIM1 expression in neonatal, adult mouse and human endometrium suggesting Lim1/LIM1 may have a role in endometrial development and remodelling.
1 Ye L et al PLoS One, 6 (6):e21136, 2011

T-047

Gene Expression with Perinatal Development in Sheep Carotid Arteries. Ravi Goyal, Nina J Chu, Dipali Goyal, Nathanael Matei, Giovanni A Longo, Lawrence D Longo. *Center for Perinatal Biology, Loma Linda University, Loma Linda, CA, USA.*
The cerebral vasculature undergoes a multitude of alterations with development from premature fetus, to near-term fetus, to newborn, to adult. Dysregulation of any of these changes may have serious consequences for the organism. Moreover, while the near-term fetus is prepared for a transition from intra- to extra-uterine life, this is not the case for the premature fetus, thus, making it more susceptible to cerebrovascular accidents. During the past several decades, others and our studies have revealed important aspects, and their differences, in the signaling mechanisms that regulate cerebrovascular contractility with maturation in the fetus and newborn, as compared to adult. The fundamental mechanisms responsible for these developmental changes are poorly understood, however. With the use of oligonucleotide microarray analysis and real-time PCR validation, we elucidated changes in the transcriptome with development in sheep carotid arteries (n = 4 for each group). Of importance, we demonstrate a U-shaped curve of gene expression during early life. For a number of major networks/pathways, the number of genes altered ($P < 0.001$) in premature fetus and newborn were 628 and 426, respectively, as compared to only 121 genes in the near-term fetus, with adult as the "gold standard". Specifically, the pre-term fetus and newborn demonstrated up-regulation of cell proliferation, growth, and assembly pathway genes, whereas MAPK-ERK, actin cytoskeleton, integrin signaling pathways were down-regulated, as compared to term-fetus and adult. Overall, in addition to elucidating important signaling pathways which undergo significant changes with maturation these studies stress the highly regulated gene expression patterns during perinatal development (Supported by HD3807 to LDL).

T-048

microRNA Expression in Granulosa Cell According to Their Oocyte Maturation during In Vitro Maturation of Mouse Follicle. Seung-Yup Ku,[1,2] Yong Jin Kim,[2,3] Yoon Young Kim,[2] Kyung Eui Park,[1,2] Seok Hyun Kim,[1,2] Young Min Choi,[1,2] Jung Gu Kim,[1,2] Shin Yong Moon.[1,2] *[1]Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, Seoul, Republic of Korea; [2]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National Univer, Seoul, Republic of Korea; [3]Department of Obstetrics and Gynecology, Maria Fertility Hospital, Seoul, Republic of Korea.*
Objective: MicroRNAs (miR) are known to repress target genes at post-transcriptional level and play important roles in development and maturation of cell. However, the expression profiles and roles of miR in granulosa cell (G-cell) during ovarian follicle maturation have not been fully elucidated. Here, we designed this study to investigate profile of microRNA and target gene expression in granulosa cells according to their oocyte maturation (MI vs. MII) and to evaluate the effect of microRNA transfection into the granulose cell during in vitro maturation of mouse ovarian follicle.
Materials and Methods: Ovaries from 2-week-old C57BL6 mice were removed and preantral follicles were isolated and cultured in 20 μL-drop of culture media with supplementation of rFSH+rLH. After ovulation was induced with adding hCG at culture day 12, granulosa cells were classified into 2 groups (MI vs. MII) according to their oocyte maturation. RNA was isolated from granulosa cells, and real-time PCR were performed with primers of microRNA known to be expressed in the mouse ovary (mmu-let-7b, mmu-miR-16, mmu-miR-126-3p, mmu-miR-143), and their candidate target genes. MicroRNAs which were suppressed in MII-granulosa cell were transfected into the in-vitro-matured follicles at ovulation-inducing time and oocyte maturation rate were compared between transfection and control group.
Results: Granulosa cells of MII expressed lower mmu-let-7b, mmu-miR-143, and higher IGF-2, BMP15, and AMH, compared to those of MI. MII rate of mmu-let-7b transfection group was lower than that of control group (14.8% vs 38.0%, $P<.05$)
Conclusions: During in vitro maturation of mouse follicle, microRNA expression in granulosa cells may be related with maturation of oocyte and its target gene expression. Transfection of microRNA into the in-vitro-matured follicles could affect their oocyte maturation (A110116).

T-049

Expression of *Tbx4* in the Developing Internal and External Reproductive Systems of the Mouse. Erica B Mahany,[1] Nataki C Douglas,[1] Kathleen Heng,[1] Mark V Sauer,[1] Virginia E Papaioannou.[2] *[1]Obstetrics and Gynecology, Columbia University Medical Center (CUMC), New York, NY, USA; [2]Genetics and Development, CUMC, New York, NY, USA.*
Purpose: *Tbx4* is a member of the T-box family of transcription factors, which is important for many aspects of embryonic development. Mutations in T-box genes are associated with developmental defects in both mice and humans. A role for *Tbx4* in sexual development has not as yet been described. In this study, we examine the spatio-temporal expression patterns of *Tbx4* in the developing mouse internal and external reproductive systems.
Methods: Embryos between E9.5 and E18.5 were collected after timed mating of wild type mice. Gonads from postnatal animals were also collected at 2 and 6 weeks of age. The sex of each embryo or postnatal animal was determined by PCR for the *Sry* gene or by morphologic differences in the gonads. At each time point, 5 males and 5 females were examined. *In situ* hybridization (ISH) was performed on whole organs or frozen sections using antisense and sense probes for *Tbx4* and other cell type-specific markers.
Results: At E11.5, *Tbx4* is expressed in bipotential gonads of male and female embryos. After sexual differentiation of the gonad at E13.5, *Tbx4* is expressed in germ cells within the testis cords and throughout the ovary. Comparisons of *Tbx4*, *Oct4*, and *Sox9* expression patterns were used to confirm germ cell specificity of *Tbx4*. *Tbx4* expression was not detected in postnatal males. In contrast, *Tbx4* is expressed in oocytes of primordial, primary, secondary and antral follicles in postnatal female mice. At E10.5 and E13.5, prior to sexual differentiation of the genital tubercle, *Tbx4* is expressed in the mesenchyme but not in the urethral epithelium. At E18.5, *Tbx4* is expressed in the mesenchyme of the penis and clitoris.
Conclusions: During embryonic development, *Tbx4* is expressed in germ cells and genital tubercles of both males and females. *Tbx4* is expressed in oocytes at all stages of follicular development. Our data sets the stage for investigating the requirements for *Tbx4* in the development of the male and female reproductive systems. As homozygous *Tbx4* null mice die embryonically, we have initiated experiments to study germ cell-specific deletion of *Tbx4* in embryos and adult mice using conditional null alleles.
Support: Reproductive Scientist Development Program (NIH K12 HD000849), Harold Amos Medical Faculty Development Program/Robert Wood Johnson Foundation

T-050

Dynamic Phosphorylation of Embryonic Poly(A)-Binding Protein (ePAB) Is Required for Oocyte Maturation. Kyle Friend,[1] Matthew Brook,[2] Betul F Bezirci,[1] Michael D Sheets,[3] Nicola K Gray,[2] Emre Seli.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine; [2]MRC Human Reproductive Sciences Unit, Queens Medical Research Institute, University of Edinburgh; [3]Biomolecular Chemistry, University of Wisconsin – Madison.*
Oocyte maturation and early embryonic development require the expression of maternally-stored mRNAs that are regulated post-transcriptionally. Embryonic poly(A)-binding protein (ePAB) is the predominant poly(A) binding protein during oocyte and early embryo development in Xenopus, mouse and human. ePAB associates with complexes containing cytoplasmic polyadenylation element binding protein 1 (CPEB1) or Pumilio2 (Pum2) that regulate mRNA expression in the oocyte. Here we report that ePAB produced in Xenopus laevis oocytes is dynamically phosphorylated and that ePAB phosphorylation determines its binding to CPEB1 and Pum2-containing complexes as well as translated mRNAs. Finally, we show that ePAB phosphorylation is necessary for oocyte maturation and mRNA cytoplasmic polyadenylation. Our findings demosnstrate that ePAB is a key regulator of oocyte development and that ePAB function is tightly controlled by phosphorylation.

T-051

In-Utero Diethylstilbestrol (DES) Exposure Downregulates HOXA10, HOXA11, and Wnt7a Expression in Human Endometrium: A Case Study. Kaitlin E Haines, Hugh S Taylor. *OB/GYN and Reproductive Sciences, Yale University, New Haven, CT, USA.*

Objective: Diethylstilbetrol (DES) is a potent synthetic estrogen that is also an endocrine disruptor; in-utero exposure results in abnormalities in female reproductive tract development. In-utero DES exposure leads to decreased HOXA10, HOXA11, and Wnt7a expression in mice, resulting in abnormal uterine development. While the effects of in-utero DES exposure on uterine gene expression have been characterized in the rodent model, they have not been confirmed in humans.

Methods: Endometrial biopsies were obtained from three women, one with a history of in-utero DES exposure and two controls. Immunostaining was performed on formalin-fixed, paraffin-embedded tissue. Primary antibodies to either HOXA10, HOXA11 or Wnt7a were diluted to 1:250 and used for immunohistochemistry. Expression was assessed by two independent observers blinded to treatment. H-Score was calculated.

Results: HOXA10, HOXA11 and Wnt7a expression was lower in endometrial tissue sections from a woman exposed to DES in-utero compared to non-exposed controls. HOXA11 expression was significantly decreased in the exposed sample; the mean H-score was 0.89 in the controls and 0.32 in the DES exposed tissue ($p<0.05$; rank sum test. HOXA10 expression was decreased 1.7 fold (Control mean H-score = 0.68, Exposed mean H-score = 0.39; $p<0.05$; rank sum test). Wnt7a expression was decreased 2.2 fold. (Control mean H-score = 1.315, Exposed mean H-score = 0.59; $p<0.05$; rank sum test).

Conclusion: We demonstrate that human endometrium exposed to DES in-utero displayed decreased HOXA10, HOXA11 and Wnt7a expression. This suggests that the mechanism by which DES alters reproductive tract development in humans is likely similar to that in mice. Despite the distinct developmental program of mice and humans, the same essential developmental genes are affected by DES exposure.

T-052

Combined Inhibition of ERK1/2 and AKT Pathways Induces Apoptosis of Human Endometriotic Epithelial and Stromal Cells through Intrinsic Apoptotic Mechanisms. Sakhila K Banu, JeHoon Lee, Robert C Burghardt, Joe A Arosh. *Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA.*

Apoptosis is a process of programmed cell death controlled by multiple cell signaling pathways. Human ectopic endometria is resistant to apoptosis compared to eutopic endometria, however; the underlined molecular and cellular mechanisms are largely unknown. Medical strategies to intervene antiapoptotic or proapoptotic pathways may lead to identify effective treatment modalities for the treatment of endometriosis in women. The remarkable redundancy of signaling pathways in the pathogenesis of endometriosis indicate that inhibition of a single ERK1/2, AKT, NFkB or b-catenin pathway could be compensated easily by other linear pathways and that could promote uninterrupted survival of endometriosis. The objective of the present study was to determine effects of inhibition of ERK1/2 and AKT pathways on survival of human endometriotic epithelial cells 12Z and stromal cells 22B. The 12Z and 22B cells were derived from active red peritoneal endometriosis lesions from women. ERK1/2 and AKT pathways were inhibited using selective inhibitors (UO1026) and (LY294002), respectively. Effects of inhibition of ERK1/2 and/or AKT pathways on survival of 12Z and 22B were determined by TUNEL and Annexin V assays and expression and interaction of proteins involved in intrinsic apoptosis pathways were determined by Western blot and immunoprecipitation. Results indicated that inhibition of either ERK1/2 or AKT induced apoptosis in 20% of 12Z and 22B cells, whereas; combined inhibition of ERK1/2 and AKT induced apoptosis in 45-50% of 12Z and 22B cells. Further, our data indicated that inhibition of both ERK1/2 and AKT pathways highly decreased ($P<0.05$) phosphorylation/activation of c-fos, c-jun, c-myc, EGR-1, and CREB proteins and induced ($P<0.05$) cleavage of caspase-3 and PARP proteins in 12Z and 22B cells. Surprisingly, inhibition of either ERK1/2 or AKT pathways produced only moderate or no effect on activation of these proteins. These results suggest that combined inhibitions of ERK1/2 and AKT pathways produced synergistic effects and required to inhibit survival of endometriotic cells 12Z and 22B. Combined targeting ERK1/2 and AKT pathways might emerge as potential non-steroidal treatment option for endometriosis in women.

T-053

Selective Inhibition of Prostaglandin E2 Receptors EP2 and EP4 Inhibits Adhesion of Human Endometriotic Epithelial and Stromal Cells through Integrin-Mediated Mechanisms. Joe A Arosh, JeHoon Lee, Roberet C Burghardt, Sakhila K Banu. *Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA.*

Endometriosis is a chronic inflammatory disease of reproductive-age women. Adhesion of endometriotic cells to peritoneum is mediated through interactions between integrin and extracellular matrix (ECM) protein. In endometriosis patients, concentrations of PGE2 in the peritoneal fluid are higher compared to that of endometriosis-free women, and this increased PGE2 is thought to promote invasion and proliferation of endometriotic cells through multiple signaling-cross talk involving ERK, AKT, NFkB and b-catenin pathways. However, PGE2 mediated molecular and cellular mechanisms that control of expression and activity of integrins and their interactions with peritoneal ECM proteins are not known in the pathogenesis of endometriosis. The objective of the present study was to determine functional interaction between PGE2 signaling and adhesion of human endometriotic epithelial cells 12Z and stromal cells 22B to ECM and to unravel the underlying molecular and cellular mechanisms. The immortalized 12Z and 22B cells were derived from active red peritoneal endometriosis lesions from women, which produce large amounts of PGE2 and express EP2 and EP4 receptors. EP2 and EP4 receptors were inhibited using pharmacological inhibitors (AH6809-75 mM and AH23848-50mM) and siRNA. Effects of selective inhibition of EP2 and EP4 on adhesion of 12Z and 22B cells to ECM and expression and activation of integrin signaling proteins were determined. Results of the present study indicate that selective inhibition of PGE2 receptors EP2 and EP4 decreases expression of integrin receptor b1 and b3 but not expression of a2, a3, a5, and av subunit proteins, decreases expression of focal adhesion kinase (FAK) and talin proteins, inhibits interactions among b1/b3, FAK, talin, and EP2/EP4 through b-arrestin-1 and Src kinase protein complex, and decreases adhesion of 12Z and 22B cells to collagen I, collagen IV, fibronectin, and vitronectin in an epithelial-stromal cell specific manner. Data from the present study for the first time provide a direct molecular link between PGE_2 signaling and adhesion of endometriotic cells to ECM in human. Results of the present study along with our previous reports suggest that inhibition of EP2/EP4 could be a potential non-steroidal treatment option for endometriosis.

T-054

FOXL2 Expression in Human Endometrium and in Endometriosis. Patrizia Carrarelli,[1] Laura Governini,[2] Ana Luiza L Rocha,[1] Romina Novembri,[1] Alice Luddi,[1] Paola Piomboni,[2] Louise M Bilezikijan,[3] Felice Petraglia.[1] *[1]Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy; [2]Biomedical Sciences, Applied Biology, University of Siena, Siena, Italy; [3]Clayton Foundation Laboratories for Peptide Biology, Salk Institute for Biological Studies, La Jolla, CA, USA.*

The FOXL2 (forkhead box L2) protein is a subclass of the winged helix of Forkhead box (FOX) class of transcription factors, essential during early ovarian development, follicle maturation, female sex determination as well as in female fertility.

In the present study, by quantitative Real Time-PCR, the FOXL2 mRNA expression in healthy human endometrium, in eutopic and ectopic tissue from women with endometriosis was investigated. Moreover, the possible correlation between FOXL2 expression and activin βA (INHBA) and follistatin (FST) expression profile in the same specimens was studied.

Endometrial and endometriotic samples were collected from a group of healthy (n=52) and endometriotic (n=41) non-pregnant women, during proliferative and secretory phases of the menstrual cycle.

By sequence verification, the expression of the FOXL2 gene in human endometrium was confirmed. In healthy women, FOXL2 mRNA expression significantly changed throughout the endometrial cycle. In particular, an higher expression of FOXL2 mRNA in late proliferative phase (0.8 ± 0.25) than in early secretory (0.61 ± 0.2) and late secretory (0.21 ± 0.14) phases, compared to the early proliferative phase ($p<0.05$) was found.

Endometriotic lesions showed FOXL2 mRNA expression levels about three fold higher than those in eutopic endometrium of endometriotic women (0.8 ± 0.24 vs 2.75 ± 0.31) ($p<0.001$).

A positive correlation between the endometrial expression of FOXL2 and INHBA or FST mRNAs was observed in healthy and in endometriotic patients ($p<0.01$).

This is the first evidence that FOXL2 mRNA is present in human endometrium. Our findings support the possibility that the expression of transcriptions factors

such as FOXL2 are modulated by menstrual cycle changes and activated in endometriotic lesions. The correlation with INHBA and FST suggest a growth factor modulatory function.

T-055

β-Fibrinogen as a Diagnostic Marker in Uterine Flushings (UF) of Baboons with Endometriosis. Asgerally Fazleabas,[1] Susan Ferguson,[1] Maria Warren.[2] *[1]Ob/Gyn & Reprod Biol, Michigan State University, Grand Rapids, MI, USA; [2]Michael Hooker Proteomics Core, University of North Carolina, Chapel Hill, NC, USA.*

The lack of a reliable biomarker for endometriosis results in patients not being diagnosed for an average of 8-11 years, and diagnosis still depends on laparoscopy. Several recent studies have identified proteomic profiles associated with the disease, but consensus between studies remains a concern. The baboon model of induced endometriosis provides a mechanism by which proteomic changes as a direct consequence of the disease can be identified. UF were collected from controls and baboons with endometriosis sequentially at 1,3,6 and 9 m of disease (n=3). The samples were prepared for 2D-DIGE. An aliquot (70 ug) from UF from animals with endometriosis were labeled with 1 of 2 fluorescent dyes (Cy3 and Cy5). A sample containing equal amounts (430 µg) of the two controls was pooled and four 70 ug aliquots were labeled with Cy2 creating a total of 4 mixtures. The mixtures were applied to isolectrofocusing strips for 1st dimensional separation and then separated by SDS-PAGE in the 2nd dimension. Fluorescently labeled proteins were imaged and UF from later time points were compared with earlier time points and controls. A protein spot (~47kD) regressively decreased as the disease progressed. Using Sypro Ruby fluorescence, the spot was excised and in-gel digested with trypsin and analyzed by MALDI-TOF/TOF. The acquired spectra were searched against a MSDB data base which identified the spot as a beta-fibrinogen fragment. To confirm the specificity of beta-fibrinogen, UF (20ug) obtained from 5 baboons before and at various times points (1 to 15 m) after induction of the disease were subjected to Western Blot analysis using an antibody against human fibrinogen (Dako – 1:100). The corresponding 47kD band was markedly decreased in UF at 6 m onwards. A similar decrease was observed in UF from baboons with endometriosis during the proliferative stage and with spontaneous disease. These data suggest that proteomic analysis of UF is a useful tool for identification of biomarkers for endometriosis and that identification of a single marker is feasible. Current studies are focused on confirming the specificity of our findings in UF from women using a beta-fibrinogen specific antibody. (U54HD40093)

T-056

Epigenetic Modifications in the Eutopic Endometrium (EUE) of Women with Endometriosis and Infertilty. Asgerally Fazleabas,[1] Niraj Joshi,[1] Mouli Gadisetti,[1] Jing Chen,[3] Shuk-Mei Ho,[2] Bruce Lessey. *[1]Ob/Gyn & Reprod. Biol., Michigan State University, Grand Rapids, MI, USA; [2]Environmental Health, University of Cincinnati, Cincinnati, OH, USA; [3]Obstetrics and Gynecology, Greenville Hospital Systems, Greenville, SC, USA.*

Endometriosis is possibly an epigenetic disease and this may contribute to the aberrant hormonal and immunological alterations associated with the disease. A number of specific genes i.e., PR and HOXA10 have been reported to be epigentically modified as a consequence of endometriosis. To determine if global epigenetic changes also contribute to endometriosis associated infertility, mid-secretory EUE from control (C), fertile (IE) and infertile (NOI: n=5/grp) women with endometriosis, were analyzed for genome-wide changes in promoter methylation patterns and compared with global gene expression profiles in the same samples to identify the effects of methylation on gene expression. The initial methylation analysis resulted in substantial changes in global methylation patterns associated with endometriosis and infertility. When correlated with the expression array, the NOI and C groups clustered separately and 140 differentially expressed transcripts ($p<0.01$: 89 up and 51 down) were identified which showed opposite changes in promoter methylation status. In contrast, IE versus C comparisons only identified 33 such genes, although in the methylation array alone the IE and NOI groups shared common methylation patterns. Ingenuity pathway analysis of the differentially expressed genes identified the arylhydrocarbon receptor pathway as one of the major pathways that was altered in the NOI group. AHR itself was hypomethylated and significantly overexpressed in NOI samples compared to controls. QRT-PCR further validated these findings and the EUE of women with endometriosis showed a higher expression ($p<0.05$) of AHR as well as its downstream targets CYP1A1 and CYP1B1. These data demonstrate that epigenetic changes in the EUE of NOI contribute to their infertility. Therefore, assessment of AHR together with the altered methylation status of the other 140 genes identified in this study may provide a diagnostic tool to identify the subset of women who suffer from infertility as a consequence of endometriosis. (U54HD40093).

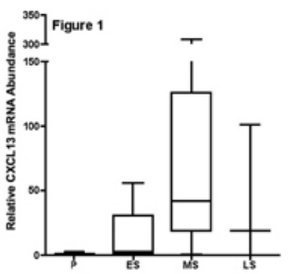
T-057

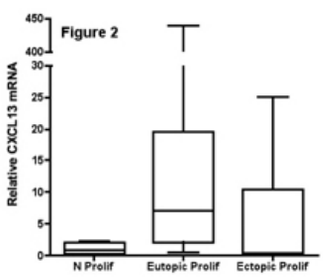
Endometrial CXCL13 Expression Is Cycle Regulated and Aberrant in Endometriosis. JF Franasiak,[1] BA Lessey,[2] L Yuan,[1] MA Fritz,[1] SL Young.[1] *[1]Ob/Gyn, UNC School of Medicine, Chapel Hill, NC, USA; [2]Ob/Gyn, Greenville Hospital System, Greenville, SC.*

Background: CXCL13 is a regulator of innate mucosal immunity and is abundantly secreted by human endometrium. Previous work also suggests specific CXCL13 uptake by human blastocysts with high implantation potential. We have shown CXCL13 expression to be a highly sensitive marker of endometrial progesterone action. Based on these findings, we hypothesized that endometrial CXCL13 expression would be markedly increased in the the mid-secretory phase and altered in women with endometriosis.

Study Design: Relative CXCL13 mRNA expression was determined by real-time RT-PCR analysis of human endometrial samples from both infertile endometriosis subjects (n=15) and normal controls (n=40). CXCL13 protein was immunohistochemcally localized in endometrial samples from normal women across the cycle using polyclonal antisera (AF801, R&D Systems). All samples were obtained under IRB approved protocols.

Results: In normal volunteers, endometrial CXCL13 mRNA expression was increased an average of 84-fold between proliferative and mid-secretory phases with intermediate expression in the early and late secretory phases (Figure 1). CXCL13 protein immuno-localized predominately to glandular epithelium and endothelium, with scattered stromal cell staining. In proliferative phase women with endometriosis, eutopic endometrial CXCL13 mRNA expression was elevated an average of 62-fold, while ectopic lesions showed a smaller increase (Figure 2). Five of eight proliferative phase subjects with endometriosis demonstrated CXCL13 expression 2 -197 times the highest level detected in eleven normal subjects. In the mid-secretory phase, there was a trend for lower CXCL13 expression in women with endometriosis.





Conclusion: 1. Endometrial CXCL13 expression is maximal in the mid-secretory phase and minimal in the proliferative phase, consistent with upregulation by progesterone and a possible role in embryo implantation. 2. CXCL13 expression is highly elevated in the proliferative phase in women with endometriosis, suggesting that endometrial CXCL13 expression might serve as a clinical marker for disease.

T-058

A Pilot Study on the Use of Andrographolide To Treat Symptomatic Adenomyosis. Xishi Liu, Sun-Wei Guo. *Gynecology, Shanghai OB/GYN Hospital, Shanghai, China.*

Background: Adenomyosis is a difficult disease to manage. We have shown previously that NF-κB is constitutively activated in adenomyosis, and that suppression of NF-κB activation results in decreased expression of COX-2, VEGF, and tissue factor, the three genes known to be involved in adenomyosis. Andrographolide (Andro) is an active ingredient extracted from Andrographis, which has been used as a medicinal herb in traditional Chinese medicine for thousands of years and is shown to be an NF-κB inhibitor. Our recent animal study shows that treatment with Andro results in retardation of myometrial infiltration, reduction of uterine contractility and contractile irregularity, and alleviation of generalized hyperalgesia in mice with induced adenomyosis.

Objective: To examine as whether Andro has any therapeutic effect in treating symtomatic adenomyosis.

Methods: Twenty-four women with ultrasonographically confirmed adenomyosis (excluding endometriosis) were recruited for this study. Most of these patients had moderate-to-severe dysmenorrhea, as reflected by the median visual analog scale (VAS) of 5.5 on a 0-10 scale, or menorrhagia. Starting at the 5th day of their menstrual cycle, all patients received 600 mg of Andro pill

orally t.i.d. for 3 months. The primary outcome measures were the VAS, PBAC score, and the uterus size. These measures were evaluated prior to the drug treatment, and 3 and 6 months after the treatment, respectively.

Results: All outcome measures were obtained at the end of the 3-month treatment,. Six patients came for evaluation 6 months after the treatment although all patients provided their VAS score over the phone. The median VAS score at the end of 3 and 6 months after treatment was both 3. The difference in VAS between 3-month evaluation and the baseline and between 6-month and the baseline was significant (both P-values=0.0006). By the end of 3-month treatment, the amount of menses was also reduced significantly, and the uterine size was reduced by 13% (p=0.04).

Conclusion: Andro appears to be well tolerated and to have some therapeutic effect in treating adenomyosis.

T-059

Disturbed Endometrial Immune Environment in Endometriosis: Novel Evidence from a Flow Cytometric Study of Leukocyte Sub-Populations. Alison J Hey-Cunningham, Robert Markham, Ian S Fraser, Marina Berbic. *Obstetrics, Gynaecology and Neonatology, The University of Sydney, Sydney, NSW, Australia.*

Introduction

Certain immune cell populations are significantly altered in number within the eutopic endometrium of women with endometriosis, however very little is known about their function and specific roles in disease pathogenesis. It has been hypothesized that local immune dysfunction in endometriosis may contribute to the establishment of the disease (via a failure to clear shed and refluxed endometrial tissue) and to endometriosis-associated infertility.

Objective

The aim of this research was to examine the numbers and function of leukocyte sub-populations in eutopic endometrium in endometriosis.

Methods

Flow cytometric analysis was conducted to study leukocytes in prospectively collected endometrial samples from women with (proliferative n=5, secretory n=9, menstrual n=4) and without (proliferative n=4, secretory n=5, menstrual n=3) endometriosis. Leukocytes were quantified (CD3, CD4, CD14, CD20, CD45, CD56) and their functional properties assessed (CD80/86, HLA-DR).

Results

Between the proliferative and secretory phases of the cycle, expression of CD80/86 activation markers on CD3+CD4- T cells significantly decreased in controls (p=0.027) but not in endometriosis subjects. In the secretory phase, numbers of CD20+ B cells were significantly decreased (p=0.022) and there was a strong trend for increased numbers of CD56+CD3+ NK T cells (p=0.053) in endometriosis compared to control endometrium. Between this phase and menstruation, NK T cells decreased in numbers in the endometriosis group (p=0.037) while in controls they did not significantly change.

Discussion

This study has observed a range of disturbances of the endometrial immune environment in endometriosis, particularly around the secretory phase. Persistent T cell activation and increased numbers of NK T cells during this time may contribute to reduced endometrial receptivity to implantation in endometriosis. In the normal cycle, immune cells, including B cells as shown in this study, are recruited prior to menstruation and play roles in endometrial breakdown, clearance and repair. These processes are also altered in endometriosis, with decreased numbers of B cells.

T-060

Peripheral Blood Leukocyte Populations during the Menstrual Cycle in Women with and without Endometriosis. Alison J Hey-Cunningham, Robert Markham, Ian S Fraser, Marina Berbic. *Obstetrics, Gynaecology and Neonatology, The University of Sydney, Sydney, NSW, Australia.*

Introduction

There is mounting evidence of immunological disturbances in endometriosis at both local and systemic levels. However, most studies have focused on single cell populations and in general, current understanding of specific functional properties of these cells and the precise roles they may be playing in the pathogenesis of the disease is limited.

Objective

To study the numbers and activation status of a range of circulating immune cell populations in women with and without endometriosis at specified phases of the menstrual cycle.

Method

Peripheral blood samples were collected from women with (n=20) and without (n=14) endometriosis (laparoscopically confirmed; all phases of the menstrual cycle). Peripheral blood mononuclear cells were isolated from blood samples and labeled with antibodies for CD45 (leukocytes), CD3 and CD4 (T cells), CD14 (monocytes/macrophages), CD20 (B-cells), CD56 (NK cells), CD80/86 (antigen presenting cells [APC] and activated T cells) and HLA-DR (major histocompatibility complex II). Immune-phenotyping of peripheral leukocytes was conducted with multicolor flow cytometry.

Results

During the proliferative phase of the cycle, numbers of NK-T cells and effector T cells were significantly increased in endometriosis (p=0.014 and p=0.05, respectively). Between the proliferative and secretory phases, intensity of CD56 expression and CD80/86 expression on T cells significantly decreased in controls (both p=0.014) but not in endometriosis patients. During menstruation, numbers of lineage negative cells (dendritic cells, DCs) and the intensity of HLA-DR expression on APCs tended to increase in controls but did not change in endometriosis.

Discussion

This study provides new evidence of systemic immunological disturbances in women with endometriosis. In endometriosis, absence of the normal menstrual increases in DC numbers and APC expression of HLA-DR which are thought to promote effective targeting of shed endometrial tissue, may facilitate survival of shed endometrium. Increased numbers and persistent T cell activation in endometriosis may occur in response to continued presence of shed endometrium.

T-061

Decreased Progesterone Receptor/FK506 Binding Protein 51 in Endometriosis; In Situ Inhibition of Transcriptional Activity of Progesterone Receptor in Endometriotic Cells. Umit A Kayisli, Frederick Schatz, Emre Vatandaslar, Nehir Ocak, Charles J Lockwood. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background: In human endometriosis, eutopic and ectopic endometrium exhibit reduced progesterone (P4) responsiveness reflecting low progesterone receptor (PR) and FK506-binding protein (FKBP)52 expression. Acting as co-chaperones with hsp90, FKBP52 and FKBP51 interact with the PR ligand binding domain. Consequently, FKBP52 enhances and FKBP51 inhibits P4-initiated transcription.

Objectives: Compare FKBP51 and PR expression and ratios in normal, eutopic and ectopic endometrial stromal and glandular cells to assess their potential involvement in diminished PR responses in endometriosis.

Methods: Serial sections (5μm) from formalin-fixed, paraffin-embedded menstrual cycle-matched normal, paired eutopic and ectopic human endometrial tissues (n=8; 5 proliferative phase and 3 secretory phase) or tissues without endometriosis (n=13; 7 proliferative phase and 6 ecretory phase) were immunostained for FKBP51 and PR and immunoreactivity was assessed semi-quantitatively by HSCORE.

Results: FKBP51 HSCORES were significantly higher in ectopic endometrial stromal cells compared with matched eutopic and cycle-matched endometrium ($p<0.001$). PR HSCOREs in ectopic endometrial stromal cells were significantly lower versus their matched eutopic and cycle matched endometrium ($P<0.02$). No significant difference was found in glandular epithelial cells for either FKBP51 or PR expression among the groups. The PR/FKBP51 ratio was significantly lower in both ectopic stromal ($p<0.004$) and endometrial glandular cells ($p<0.02$) compared with that in normal and eutopic endometrium.

Conclusions: Diminished P4 responsiveness in endometriosis correlates with decreased PR and increased FKBP51 levels in ectopic endometrium. A decrease in the PR/FKBP51 ratio amplifies inhibitory effects of FKBP51 on the transcriptional activity of the PR.

This study is supported by NIH Grant U54 HD052668.

T-063

Slit2 Overexpression Results in Increased Microvessel Density and Lesion Size in Mice with Induced Endometriosis. Yuan Lu,[1] Xishi Liu,[1] Sunwei Guo,[1] Jian Guo Geng.[2] *[1]OB/GYN Hospital, Fudan University, Shanghai, China; [2]Department of Biologic and Materials Sciences, University of, Ann Arbor, Michigan School of Dentistry.*

BACKGROUND: We recently reported that SLIT/ROBO1 pathway is a constituent biomarker for recurrence of endometriosis, likely through promoting

angiogenesis. We sought to determine as whether Slit2 overexpression can facilitate angiogenesis and increase lesion size in induced endometriosis in mice.

METHODS: Thirty Slit2 transgenic (S) and 29 wild-type (W) mice were used in this study. We cross-transplanted endometrial fragments from S to W (group SW) and vice versa (group WS), and also within the S and W (groups SS and WW, respectively), inducing endometriosis. We also performed a sham surgery within both S and W mice (groups Sm and Wm, respectively). We then evaluated the size of the ectopic implants, microvessel density (MVD) and immunoreactivity to ROBO1, and VEGF in ectopic and eutopic endometrium as well as in vagina, along with hotplate and tail-flick tests in all mice.

RESULTS: The induction of endometriosis resulted in generalized hyperalgesia, which was unaffected by Slit2 overexpression. Slit2 overexpression did increase the lesion size significantly, and correlated positively with the expression of ROBO1 and VEGF and MVD in ectopic and eutopic endometrium and vagina. SLIT2 expression levels appear to be more important than that of VEGF in determining the MVD in ectopic endometrium.

CONCLUSIONS: Slit2 plays an important role in angiogenesis in endometriosis. The increased angiogenesis, as measured by MVD and also perhaps by increased VEGF immunoreactivity, likely resulted in increased lesion size in induced endometriosis. Thus, SLIT2/ROBO1 pathway may be a potential therapeutic target for treating endometriosis.

T-064

Identifying the Genes Associated with Endometriosis Risk on Chromosomes 1 and 7. Grant W Montgomery,[1] Dale R Nyholt,[1] Stuart Macgregor,[1] Jodie N Painter,[1] Hien TT Luong,[1] Marcel E Dinger,[3] Andrew P Morris,[2] Susan A Treloar,[4] Stacey A Missmer,[5] Peter AW Rogers,[6] Krina T Zondervan.[2] *[1]Genetics Division, Queensland Institute of Medical Research, Brisbane, QLD, Australia; [2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; [3]Institute for Molecular Biology, University of Queensland, Brisbane, QLD, Australia; [4]Centre for Military and Veterans' Health, The University of Queensland, Brisbane, QLD, Australia; [5]Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; [6]Department of Obstetrics & Gynaecology, University of Melbourne, Melbourne, VIC, Australia.*

Genome-wide association studies report strong evidence for association between endometriosis and genetic markers on chromosomes 1 and 7. An important step in translating these results into an improved understanding of disease mechanisms is to identify the relevant gene or genes in each region influencing disease risk. Using data from international sequencing projects we imputed SNP genotypes across each region. Following imputation, several variants in the region of *WNT4* showed stronger evidence of association with endometriosis and signals were confirmed with additional genotyping in our cases and controls. In contrast, the strongest evidence for association on chromosome 7 was with rs12700667, the original signal in this region. We sequenced pools of RNA from endometrial samples and are conducting RNA capture and sequencing of individual samples for each region to look for novel transcripts, allele specific splice variants and allelic differences in gene expression. Rare coding variants in genes within these regions in some high risk families might also point to the likely casual genes. We sequenced the coding regions of selected genes and conducted next-generation sequencing of the target regions in pools of DNA from 384 endometriosis cases and 384 controls. Sequencing of WNT4 in endometriosis cases identified a novel coding variant and an insertion/deletion in the 3'UTR, but these were not related to endometriosis risk.

T-065

Effects of Norethindrone Acetate vs Lupron Depot-3 in Suppressing Estradiol during Treatment of Endometriosis. Ozgul Muneyyirci-Delale,[1] Cassandra Charles,[1] Nanna Osei-Tutu,[1] Rudolph Parris,[1] Jenny Anopa,[1] Mudar Dalloul,[1] Vijaya L Nacharaju,[1] Jeremy Weedon,[1] Pamela Stratton.[2] *[1]OB/GYN, SUNY Downstate Medical Center, Brooklyn, NY, USA; [2]Eunice Kennedy Shriver National Institute of Child Health, NIH, Bethesda, MD, USA.*

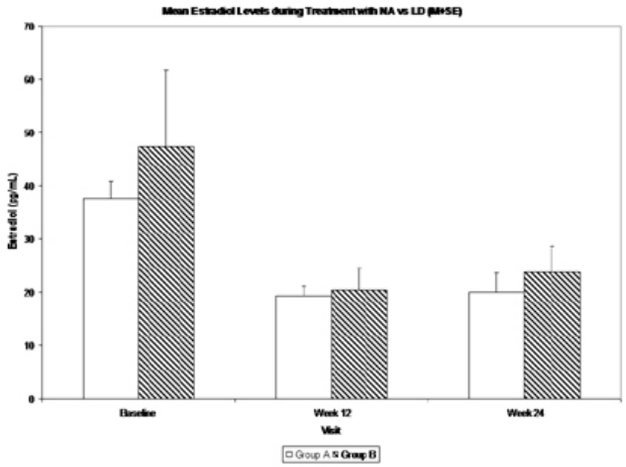
Background: Norethindrone Acetate (NA), a progestin, and Lupron Depot-3 (LD), a gonadotropin releasing hormone analogue, are both FDA-approved treatments for endometriosis with different mechanisms of action. Since the mechanisms of actions and side effects differ between the medications, we expected that estradiol (E_2) levels would subsequently vary as well.

Objective: To compare E_2 suppression by LD and NA.

Study Design: Prospective randomized double masked study.

Methods and Materials: 56 women with symptomatic endometriosis were randomized in a double-masked study of LD 11.25mg vs. NA 5mg for 24 weeks. Serum was collected at baseline then repeated at Week 12 and Week 24 study visits. E_2 levels were determined using DSL/Beckman Coulter Ultra Sensitive RIA kit. A mixed linear model was constructed for statistical analysis and Tukey-adjusted p-values are reported for post-hoc pairwise tests.

Results: Of 56 women, 27 were in group A and 29 in group B. In both study arms, mean E_2 levels fell from baseline to Week 12 ($p< 0.0001$) and remained low during treatment. E_2 did not differ between groups over time ($p=0.777$) and was significantly decreased in all women during treatment ($p< 0.0001$).



Conclusion: E_2 was significantly suppressed over time during treatment in both groups. Low E_2 is likely related to the effectiveness of each of these agents in treating endometriosis.

Support: NIH/NICHD grant # R01 HD043281-05

T-066

Gene Therapy of Abdominal/Pelvic Post-Operative Adhesions: Targeting Adenovirus towards Human Peritoneal Adhesion Cells. Sangeeta Nair,[1] Ghassan M Saed,[2] Hussain M Atta,[3] Michael Diamond,[2] Ayman Al-Hendy.[1] *[1]CWHR, Dept of Obstetrics Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [3]Department of Surgery, Minia University, El-Minia, Egypt.*

Background: Post-operative abdominal/pelvic peritoneal adhesions are a major source of morbidity and includes bowel obstruction, infertility, chronic pelvic pain as well as ectopic gestation in women. We have previously introduced the concept of using adenovirus vector encoding human tPA gene (tissue plasminogen activator) to reduce post-operative adhesion formation (Surgery 2009; 146:12-17).

Objective: To screen various transduction and transcription modifications of adenovirus with an aim to identify specific modifications that support maximal adenovirus-mediated gene delivery to human adhesion cells, which in turn would enhance the efficacy of this novel treatment/preventative strategy for post-operative adhesions.

Materials: We transduced primary cultures of human peritoneal adhesion cells with various fiber modified adenovirus vectors: Ad5-RGD-luc, Ad5-Sigma-luc, Ad5/3-luc and Ad5-CAV2-luc as well as transcriptional targeted viruses: Ad5-Survivin-luc, Ad5-Heparanase-luc, Ad5-MSLN-CRAD-luc and Ad5-SLPI-luc and compared their activity to wild type Ad5-luc at 5, 10 and 50 plaque forming units (PFU)/cell. At 48 hours, luciferase activity was measured (Promega) and normalized to the total protein content in the cells (Pierce).

Results: Among the tested fiber modified adenovirus vectors, Ad5-Sigma-luc showed the highest luciferase activity with significant increase at all tested virus concentrations at 5pfu/cell, 10pfu/cell and 50pfu/cell of Ad5-luc activity (wild type) in the adhesion cells ($P<0.05$). Among the transcription targeting modified adeno viruses, Ad5-MSLN-CRAD-luc showed the highest luciferase expression (190% and 156% at 5pfu/cell and 10pfu/cell respectively) of wild type Ad5-luc activity ($P<0.05$).

Conclusion: Adenovirus with specific modifications such as the fiber modified Ad5-Sigma-luc and transcriptional targeted Ad5-MSLN-CRAD-luc improves gene delivery efficiency towards human peritoneal adhesion cells. These modified adenoviruses may be used to develop a safe localized method to reduce/prevent post-operative adhesions in subjects.

Support: RCMI grant G12 RR 03032, USDA B1 58-3148-5-125

T-067

micoRNA-200c Is Differentially Expressed in Endometrium during Normal Menstrual Cycle, Benign and Progression into Cancerous States and Functionally Targets Inflammatory and Angiogenic Mediators. Harekrushna Panda, Leisle Pelakh, Tsaider Chuang, Xiaoping Luo, Orhan Bukulmez, Nasser Chegini. *Obstetrics and Gynecology, University of Florida, Gainesville, FL, USA.*

miRNAs have emerged as posttranscriptional gene regulators and their aberrant expression is closely associated with various disorders. In this study we explored the expression of miR-200c and a few of its predicted/validated target genes in normal endometrial biopsies (EB) from throughout the menstrual cycle (N=15), endometrial tissues (ET) from benign conditions (leiomyoma and uterine prolapsed) (N=20), and endometrial cancer (EC, Stage I-III, N=17). The results indicated that miR-200c, ZEB1, ZEB2 and their downstream gene, E-cadherin (CDH1) are expressed in all tissue examined with steady increase in the level of miR-200c expression in EBs from proliferative to late secretory phase, no significant alteration in ETs and progressive decline in EC from stage 1 into satge3. There was no correlation between the expression of miR-200C and ZEBs with the exception of EC, whereas CDH1 expression was higher in EB from mid-late proliferative and late secretory phase, with no difference in ETs and ECs. Treatment of Ishikawa cells with 10nM of 17β estradiol (E2), progesterone (P4) and medroxyprogesterone (MPA) for 6h-48h increased miR-200c level, while progressively decreased the expression of ZEB1 and ZEB2 up to 24hrs of treatments without affecting CDH1 expression. Gain-of function of miR-200c in Ishikawa cells resulted in regression of ZEBs expression (at mRNA level) as well as the expression of KLF9, IKKB, IL8, fubulin-5 (FBLN5), FLT1 and VEGF-A, as confirmed by QRT-PCR and western blot analysis and 3'UTR reporter assay. Luciferase reporter assay for 3'UTR revealed ZEBs, VEGF-A, IKKB and KLF9 as direct targets of miR-200c while IL-8, FLT1 and FBLN5 did not show any significant interaction and their expression is indirectly controlled by miR-200c. These results imply that miR-200c is expressed in endometrium during normal and diseased conditions with limited correlation with the expression of ZEBs and CDH1 with the exception of EC and through direct or indirect functional regulation of the above genes, it may influence not only epithelial to mesenchymal transformation, but also inflammatory and angiogenic pathways that are equally important in endometrial activities during normal menstrual cycle, and progression into diseased states. Supported by HD37432 and HD58664.

T-068

A Novel Approach to Quantifying Pain Perception in Women with Endometriosis: A Pilot Study. Maysa' M Khadra,[2] Kathleen M Peters,[1] Ian S Fraser.[1] *[1]Department of Obstretrics, Gynaecology and Neonatology, University of Sydney, Sydney, NSW, Australia; [2]Department of Obstetrics & Gynaecology, Jordan University Hospital, Amman, Jordan.*

Introduction

Endometriosis affects 10 – 15% of women of reproductive age. Symptomatically it may result in severe pelvic pain. Correlation between severity of disease and type of pain has not been demonstrated. Likewise, pain mechanisms are not well understood. Recent evidence shows higher densities of nerve fibres in the both the eutopic endometrium and ectopic sites of women with endometriosis suggesting a role in pain generation . The increased nociceptive input from the eutopic endometrium and ectopic sites may lead to sensitization of the nervous system and neuropathic pain. Therefore women with endometriosis and chronic pelvic pain may display increased peripheral pain sensitivity.

Objective

This study aimed to quantify peripheral pain sensitivity in women with endometriosis compared to healthy controls.

Method

In this pilot study we tested five women all with laparoscopically-confirmed endometriosis. We utilized the Quantitative Sensory Testing (QST) protocol as established for neuropathic pain (1). QST measures the detection threshold of calibrated sensory stimuli. A total of ten vibratory, painful or thermal stimuli were applied to these women.

Results

The results for each patient and each stimulus were compared to the established references values for healthy volunteers (1). The data revealed that women with endometriosis exhibited lower thresholds for most QST parameters. Interestingly, all women with endometriosis experienced Mechanical Pain Sensitivity (MPS) higher than the upper limit of the 95% Confidence Interval for the established reference range. This means they all felt pinpricks in their hands and feet more strongly than those without endometriosis.

Discussion

Psychophysical quantitative testing has demonstrated that women with endometriosis have increased pain perception. Distant peripheral sites were more sensitive to applied stimuli indicating a central hypersensitivity in these women. Improving comprehension of complex pain mechanisms may help elucidate the pathophysiology of the disease and lead to development of mechanism-targeted therapeutics.

1. Rolke R., et al. Quantitative sensory testing in the German Research Network on Neuropathic pain (DFNS): Standardized protocol and reference values. *Pain* 123:231-243

T-069

Endometriosis in Remote Settings. Edgardo Somigliana,[1] Paola Vigano,[2] Francesca Crovetto,[1] Paola Panina,[2] Alessio Paffoni.[1] *[1]Obstet-Gynecol, Fondazione Cà Granda, Milan, Italy; [2]Obstet-Gynecol, San Raffaele Scientific Institute, Milan, Italy.*

Background: The origin of endometriosis is still enigmatic. In this study, we hypothesized that the mutated reproductive patterns of women in today's affluent Western nations and in particular the reduction in the number of children per couple, the decreased duration of breast-feeding and the common habit to postpone the age at first pregnancy may play a central role in the pathogenesis of the disease. Women in Western nations are in fact exposed to an "unnatural" high number of menstrual cycles and this may favour the development of endometriosis. To verify this hypothesis, we investigated the prevalence of the disease in a rural remote setting characterized by high fertility rate, frequent teen-age pregnancy and protracted breast-feeding.

Materials and methods: The study was conducted in Oyam, a rural district localized in Northern Uganda. In this area, the total fertility rate is 7.1 children per women, mothers typically become pregnant for the first time at the age of 13-15 years and they breast-feed for up to two years. From October 01st 2009 to September 30th 2010, all cases referred to the Outpatient Department of the District Hospital for a gynecologic consultancy were consecutively and prospectively evaluated by a single gynecologist. A woman was considered to have endometriosis if she had a history of surgery for the disease or if she had a positive clinical or ultrasound examination.

Results: A total of 528 gynecological consultancies were performed during the study period. Endometriosis was recorded in one case. The frequency of the disease in the whole cohort of referred cases was thus 0.2% (95%CI: 0.01-0.9%). When focusing on non-pregnant women in their reproductive age (n=351), it was 0.3% (95%CI: 0.01-1.3%). When considering women complaining symptoms suggestive for endometriosis (n=248), it was 0.4% (95%CI: 0.02-1.9%).

Conclusions: Endometriosis was rare in a community characterized by high fertility rate, frequent teen-age pregnancy and protracted breast-feeding supporting the view that the modern mutated reproductive pattern of women in the Western World could play a crucial role in the pathogenesis of the disease.

T-070

Characterization of Immune Cell Populations in Murine Endometriosis-Like Lesions. Aleksandar K Stanic, Jill A Attaman, Minji Kim, Aaron K Styer, Bo R Rueda. *Vincent Center for Reproductive Biology, Vincent Department of Obstetrics and Gynecology, Massachusetts General Hospital/Harvard Medical School, Boston, MA.*

Background: The population dynamics and activation state of innate and adaptive immune cells in endometriosis lesions has not been extensively characterized. Given the proposed role of inflammation during the early establishment, angiogenesis, and proliferation of the disease, characterization of the ambient intralesion immune cells may offer insight into the pathogenesis of endometriosis. Objective: The objective of this study was to investigate the composition and activation state of immune cells infiltrating endometriosis-like lesions in a murine model of endometriosis. Methods: We utilized a uterine tissue transplant model with immunocompetent 8-10 week old C57BL/6 female sibling mice. Uterine tissue of donor mice was primed with a subcutaneous estradiol (E2) injection 4 days prior to harvesting and transplanted intraperitoneally into an E2-implant bearing host mouse. Mice were sacrificed 7 days later and endometriosis-like lesions (lesions) and spleen controls were collected, dissociated into single cell suspensions and labeled with fluorescent antibodies specfic for CD3, CD4, CD8, CD11c, CD31, CD40, CD45, C69, CD80, CD86, CD146, F4/80, Tcrß, Ly6G, B220, and Epcam. Fluorescence was quantified by 10-channel multiparametric flow cytometry on a 3-laser LSR II instrument. Mean fluorescence intensity of activation markers (CD69, CD80 and CD86) was determined on gated immune cells and compared between

lesions and quiescent splenocytes. Statistical significance was determined by unpaired two-tailed Students t-test. Results: T cells, B cells, dendritic cells (DCs), macrophages, and granulocytes were identified in all lesions, comprising in aggregate about 50% of all mononuclear cells isolated. DCs and granulocytes in lesions were activated with significantly ($p<0.05$) higher levels of co-stimulatory molecules CD80 and CD86 compared with quiescent splenic DCs and granulocytes. T and B cells responded by upregulation of early activation marker CD69 ($p<0.05$). Conclusion: These results indicate that activated immune cell infiltration comprises a large proportion of all isolated cells in endometriosis-like lesions. This novel model system will assist with future comprehensive study of immunocyte function in endometriosis-like lesions in order elucidate the role of immune system in the human disease.

T-071

Endometrial Cell Motility in Endometriosis under Hypoxic Microenvironment. CW Tan,[1,2] YH Lee,[1] CP Ng,[1] HH Tan,[3] SF Loh,[3] M Choolani,[2] L Griffith,[4] J Chan.[1,2,3,5] *[1]BioSym, Singapore-MIT Alliance for Research & Technology; [2]Yong Loo Lin School of Medicine, National University of Singapore; [3]Dept. Reproductive Medicine, KK Women&Children Hospital; [4]Dept. Biological & Mechanical Engineering, MIT; [5]Cancer & Stem Cell Biology Program, Duke-NUS Graduate Medical School.*

Introduction: The aetiological origin of endometriosis has remained controversial,with the effects of the local cellular environment on endometrial cells on lesion initiation being an area of investigation.Previous reports have shown that the expression of hypoxia-inducible-factor-1(HIF-1)was significantly higher in endometriosis compared with controls(Ren et al. 2007).Besides, an increased expression of focal adhesion kinase(FAK)in endometriotic tissue also alluded to altered cell motility in endometriosis(Mu et al. 2008), yet there is little known about the relationship between hypoxia and cellular migration in the pathogenesis of such lesions.

Objectives:To examine the migratory behavior of endometrial stromal cells(ESCs) derived from the eutopic endometrium in patients with endometriosis versus healthy subjects when they are exposed to a hypoxic environment.

Methods:Endometrial curettage samples were obtained from women with endometriosis(Stage IV AFS) or other benign gynecological diseas(Stage 0 AFS) undergoing laparoscopic surgery. ESCs were isolated by enzymatic digestion and sedimentation, followed by magnetic sorting with CD10 and CD45 for stromal cell purification free of leukocytes. At passage 1,cells were exposed to hypoxia(2% O_2) and assayed for cellular motility with the use of boyden chamber, RT-PCR and ORIS cell migration invasion assay.

Results:Flow cytometric analysis of ESCs derived from both cases and controls expressed CD10(>90%) and were negative for CD45(<10%).ESCs derived from patients with endometriosis were observed to migrate more aggressively than control ESC,and exhibit a heightened invasive phenotype especially under hypoxic condition.This was confirmed through immnunostaining of ESCs with vinculin and phalloidin in terms of cytoskeletal distribution.

Conclusion:The CD10+ESCs derived from eutopic endometrium of women with endometriosis showed altered cell migratory behavior as compared to those from healthy women especially when these cells are being exposed to hypoxic conditions.More investigations will be done on the key regulators in hypoxic condition to delineate the mechanism on altering the cell motility.

T-072

Severity of Dysmenorrhea Is Correlated with Overall Elevated Pain Report. Allyson Westling,[1] James Griffith,[2] Julia Resnick,[1] Kevin Hellman,[1,3] Frank Tu.[1,3] *[1]Ob/Gyn, NorthShore University HealthSystem, Evanston, IL, USA; [2]Medical Social Sciences, Northwestern University, Chicago, IL, USA; [3]Ob/Gyn, University of Chicago, Chicago, IL, USA.*

Objective: Dysmenorrhea remains a poorly understood risk factor for chronic pelvic pain (CPP). One in every five women with menstrual pain will develop a CPP disorder such as endometriosis, interstitial cystitis, or irritable bowel syndrome, but menstrual pain has largely been ignored as a potential target for risk reduction. As part of a broader survey, we investigated the relationship of dysmenorrhea to 7 CPP-like pains: bladder, intercourse, urination, bowel, abdominal, pelvic, and overall pain categories.

Methods: A web-based online assessment of non-pregnant women (age 18-45) was administered. Demographic questions and menstrual timing questions were queried, as well as items addressing pertinent constructs based on prior literature and focus group results: fatigue, mood, pain, somatic symptomatology, and physical functioning. Multivariable linear regression was used for statistical evaluation.

Results: Among 1021 participants, mean age was 35.06 years old (SD 7.78), with an average parity of 1.3 (SD 1.4). Over 70% of the women reported having a regular, 21-35 day interval menstrual cycle, with the typical menses lasting 5 days (SD 1.54). Over 40% of these subjects indicated moderate or worse dysmenorrhea, and 10% had severe dysmenorrhea. The overall VAS pain score among this cohort at the time of the survey was 2.4 (SD 2.57). The reported severity of dysmenorrhea and the seven different types of CPP-like pain symptoms were significantly correlated (R^2=0.19). Only a weak relationship exists between reported pain intensity in these seven pain categories and being on the menses at time of assessment (R^2=0.01), suggesting that the severity of dysmenorrhea is associated with regional pain sensitivity throughout the entire menstrual cycle. Multivariable linear regression identified that a combined model that includes anxiety and dysmenorrhea, but not depression best predicts CPP severity (R^2=0.35).

Discussion: Self- reported intensity of abdomino-pelvic pain in a general population sample of menstruating women is positively associated with dysmenorrhea intensity. Given the well-known phenomenon of cross-organ sensitization, earlier identification of women with more severe dysmenorrhea may permit pre-clinical detection of future CPP patients, opening the door to prevention.

T-073

Diagnostic Accuracy of CA 125 Modifications throughout Menstrual Cycle and after GnRH-Analogue Administration To Identify Endometriosis as Cause of Chronic Pelvic Pain. Roberta Venturella, Rita Mocciaro, Angela Sacchinelli, Michele Morelli, Fulvio Zullo. *Obstetrics & Gynecology, University Magna Graecia of Catanzaro.*

Background: Endometriosis is the first single cause of chronic pelvic pain (CPP). To date, the gold standard to diagnose it is the histological confirmation of lesions obtained by surgery. However, a non invasive cheaper tool for early diagnosis is strongly needed.

The aim of this study is to evaluate the diagnostic accuracy to detect endometriosis of serum CA125 modifications throughout menstrual cycle, and to analyze whether the variations of CA125 levels after one dose of GnRH-analogue (GnRH-a) may increase the diagnostic value of the assay.

Design: Prospective controlled study.

Patients: 84 women scheduled for a diagnostic laparoscopy for CPP (group A) and other 12 women scheduled for diagnostic laparoscopy for unexplained infertility (group B) were enrolled as cases and controls.

Interventions: Serum CA125 was determined for each patient at early follicular and luteal phases. Before surgery, patients from group A received one vial of leuprolide acetate depot. After laparoscopy and histological examination, cases were sub-grouped in group A1 (subjects with endometriosis) and group A2 (subjects without endometriosis).

Main outcomes: Diagnostic accuracy calculated with a ROC curve and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and relative risk (RR) of CA125 modifications throughout menstrual cycle and after GnRH-analogue administration.

Results: Plasma CA125 levels during the early follicular phase were significantly higher than that recorded in the luteal phase in all groups and they were significantly higher in group A1 in comparison with groups A2 and B. One month after GnRH administration, a significant reduction in plasma CA125 levels was observed only in group A1. The ROC analysis was significant (AUC=0.72 and 0.79, $P<0.05$), with a cut point of 40.5 and 27.6 for delta early follicular phase vs. after GnRH-a, and for delta early follicular phase vs. luteal phase, respectively. By combining these two delta, the highest sensitivity (87.0%), specificity (54.2%), PPV (50.1%), NPV (94.6%), and RR (4.6) for detecting patients with endometriosis were observed.

Conclusions: The evaluation of serum CA125 modifications throughout menstrual cycle and after one dose of GnRH-a demonstrated a good diagnostic accuracy to detect endometriosis in patients with CPP.

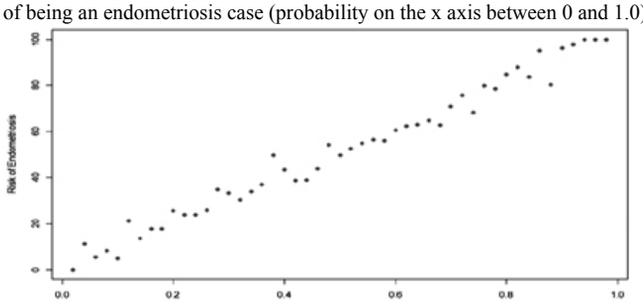
T-074

Non-Invasive Determination of a Patient's Risk of Having Endometriosis: Genetic Markers and a Clinical History Provide Actionable Likelihood Ratios. Kenneth Ward, Rakesh Chettier, Pam Farrington, Hans Albertsen. *Research, Juneau Biosciences, Salt Lake City, UT, USA.*

In Obstetric care, maternal serum screening (based on multiple serum markers and a few clinical parameters) currently guides the use of invasive diagnostic procedures (genetic amniocentesis). We sought to develop panel of genetic markers and clinical factors to guide use of laparoscopy in managing endometriosis.

Methods: 300 Caucasian patients with proven endometriosis were compared with 300 population controls with no history of endometriosis. In this analysis, we assumed that controls were unaffected even though 5 to 10% may have undiagnosed endometriosis. Prior probability of having endometriosis was calculated for each individual based on answers to simple questions (age, race, parity, age at menarche, history of dysmenorrhea, and family history of endometriosis). Conditional probability was calculated based on the genotypes for 54 DNA markers (selected as the most-promising markers from our recent GWAS). Stepwise logistic regression was used to weight the markers using a training set of 1600 cases and 1024 controls. No interaction terms were allowed. Bayesean methods were used to calculate a post-test likelihood of endometriosis.

Results: The genetic algorithm showed a linear correlation with the probability of being an endometriosis case (probability on the x axis between 0 and 1.0).



When individual prior probability was also considered, discrimination of cases and controls improves. Over 99% with scores in the top decile and 95% of subjects in the top 3 deciles had endometriosis.

Conclusions: The predictive DNA algorithm shows excellent promise as a screening test. Inclusion of clinical and demographic risk factors helps to account for environmental factors and unmeasured genetic factors. The algorithm may be further improved by considering interaction terms and biologic pathways. Eventually, a genotype panel may provide better information to guide patients and physicians as they decide whether to consider surgical management. It is also possible that describing "genetic sub-types" that may enable more individualized approaches to care.

T-075

Urinary Proteomics To Identify Screening Biomarkers in Endometriosis. Fred TK Wong,[1] Cecilia HM Ng,[1] Ben Crossett,[2] Robert Markham,[1] Ian S Fraser.[1] *[1]Obstetrics, Gynaecology and Neonatology, Queen Elizabeth II Research Institute for Mothers and Infants, The University of Sydney, Sydney, NSW, Australia; [2]School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia.*

Background

Timely diagnosis of endometriosis is challenging, 10% of women in their reproductive years, suffering endometriosis, may experience debilitating pain, adverse social and economic disadvantages. To date, full elucidation of pathogenesis and identification of biomarkers with sufficient sensitivity and specificity remains elusive. The current "gold-standard" for diagnosis of endometriosis remains laparoscopy.

Objective

This study aimed to further refine the methodology of urinary proteomics in the search for potential screening biomarkers for endometriosis. Arising from an initial study and preliminary finding of cytokeratin 19 as a potential urinary biomarker for endometriosis, we have aimed to mine deeper into the urinary proteome to identify lower abundance proteins and to understand key molecular networks involved in the pathogenesis of endometriosis.

Methods

Urine samples collected (with a protease inhibitor) from women laparoscopically diagnosed with and without endometriosis. Solubilised protein extracts, were subjected to pre-fractionation (Zoom IEF Fractionator, Invitrogen) according to the proteins' isoelectric point. Each fraction (pH 4-5, 5-6 and 6-7) collected was subjected to 2D gel electrophoresis (2DE). Resulting gel image analysis detected differential protein abundance using Progenesis SameSpot software (NonLinear Dynamics). Proteins of interest were identified by peptide mass fingerprinting using MALDI-TOF mass spectrometry (Voyager, AB Sciex, Australia) and MASCOT (Matrix Science, UK).

Results

Initial proteomic analyses are promising, indicating that 133 spots are differentially expressed between the fractions in women with and without endometriosis. Many of these appear to be cell structural proteins. Further testing is currently being carried out to characterise these proteins of interest.

Conclusions

These promising preliminary results indicate that pre-fractionation coupled with 2DE offers greater protein separation capabilities enabling vision of lower abundance proteins. Reducing the complexity of the sample of interest. This provides evidence that urinary proteomics may allow the identification of biomarkers suitable for screening in women suspected of endometriosis.

T-076

FKBP52 Is Regulated by HOXA10 in Decidualization and in Endometriosis. Huan Yang, Yuping Zhou, Benjamin Edelshain, Frederick Schatz, Hugh S Taylor. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: FKBP52 enhances and FKBP51 inhibits progestin-mediated transcription by the progesterone receptor (PR). Here we examined the effects FKBP52 and FKBP51 on the induction and maintenance of decidualization of human endometrial stromal cells (HESCs) and assessed HOXA10 regulation of FKBP52.

Design: Expression of FKBP52 and FKBP51 mRNA was examined in eutopic endometrium from control women and from subjects with endometriosis. Cultured HESCs were treated with either FKBP52 or FKBP51 siRNA then decidualized by incubation with progesterone (P4) and 8-Bromoadenosine 3', 5'-cyclic monophosphate (cAMP). Decidualization was monitored by induction of insulin-like growth factor binding protein-1 (IGFBP-1). Regulation by HOXA10 was determined after treatment with HOXA10 siRNA.

Results: Expression of FKBP52 mRNA was increased in the late proliferative phase and remained elevated through the secretory phase. FKBP51 expression was low and remained constant throughout the menstrual cycle. Compared with controls, FKBP52 mRNA expression was decreased in eutopic endometrium from women with endometriosis across the menstrual cycle, whereas no significant endometriosis-related change was seen for FKBP51. FKBP52 siRNA treatment of HESCs resulted in 60% lower IGFBP-1 expression, whereas incubation with FKBP51 siRNA did not significantly affect IGFBP-1 expression during *in vitro* decidualization. HOXA10 and FKBP52 expression increased in parallel during in vitro decidualization. Over-expressed HOXA10 enhanced FKBP52 mRNA levels by 3-fold, whereas HOXA10 knock-down decreased FKBP52 mRNA to 36% of control levels. Western blotting confirmed that HOXA10 regulates FKBP52 protein expression in HESCs. During *in vitro* decidualization, FKBP52 expression was decreased in HOXA10 silenced cells.

Conclusion: Enhanced HOXA10 expression in HESCs elicits a decidualization mediating increase in FKBP52 expression. These *in vitro* observations are consistent with a report in patients with endometriosis showing that down-regulation of FKBP52 expression in eutopic endometrium impairs decidualization and leads to infertility. Consequently, progesterone resistance seen in endometriosis may reflect a decrease in HOXA10 mediated by FKBP52.

T-077

FKBP51 Expression in Ectopic and Eutopic Endometrium in Women with Adenomyosis. Chih-Feng Yen,[1] Frederick Schatz,[2] Charles J Lockwood,[3] S Joseph Huang.[2] *[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [3]Obstetrics and Gynecology, Ohio State University, Columbus, OH, USA.*

Context: Adenomyosis, identified by endometrial glands and stroma in the myometrium, is an estrogen-dependent condition with ubiquitous estrogen receptors and reduced progesterone preceptors (PR). The FK506-binding proteins, FKBP51 and FKBP52, act as co-chaperones with heat shock protein 90 (hsp90) to interact with the ligand-binding domain of the PR. Consequently, FKBP51 inhibits and FKBP52 enhances progestin-initiated transcription.

Objective: Determine relative abundance of FKBP51 and FKBP52 in glandular epithelial cells (GEC) and stromal cells (SC) by immunolocalization in adenomyosis sections and functional layer from eutopic endometrium obtained across the menstrual cycle.

Methods: Biopsies of eutopic endometria and adenomyosis from patients as well as control endometrium (CON) from patients with myoma across menstrual cycle were obtained. Two-micrometer formalin-fixed and paraffin-embedded sections were immunostained using primary monoclonal goat and mouse anti-human antibodies against FKBP51 and FKBP52, respectively, followed by FITC-conjugated anti-goat and IgG Rodamine-conjugated anti-mouse secondary antibodies. Relative abundance of FKBP51 and FKBP52 was determined by evaluating individual fluorescence intensity.

Results: All sections examined displayed excess FKBP51 versus FKBP52 thus limiting further evaluation to FKBP51. Tables 1 & 2 summarize the results.

Conclusions: FKBP52 potentiates, whereas progestin- FKBP51 reduces progestin responsiveness. This study reveals dominance of FKBP51 over FKBP52 in nuclei of glands and stromal cells in all tissues and a different expression pattern of FKBP51 in CON compared with that in eutopic tissue and adenomyosis from patients. Since FKBP51 is a nuclear factor that promotes progestin resistance, these findings may help to develop potential therapies to treat adenomyosis via enhanced progestin action.

Comaprison of FKBP51 Localizations

Tissue	Cell Type	Proliferative		Secretory	
		Cytoplasm	Nuclei	Cytoplasm	Nuclei
CON	GEC	+	-	-	+
	SC	-	+	-	+
Eutopic	GEC	+	+	+	+
	SC	-	+	-	+
Adenomyosis	GEC	+	+	-	+
	SC	-	+	-	+

Comparison of FKBP51 Levels

Tissue	Proliferative	Secretory
CON	GEC < SC	GEC ~ SC
Eutopic	GEC > SC	GEC > SC
Adenomyosis	GEC > SC	GEC > SC

T-078

Raf-1 Levels Determine the Migration Rate of Primary Endometrial Stromal Cells of Patients with Endometriosis. Iveta Yotova, Ping Quan, Aulona Gaba, Nadja Leditznid, Petra Pateisky, Christine Kurz, Walter Tschugguel. *Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria.*

Introduction: Endometriosis is a disease characterized by the localization of endometrial tissue outside of the uterine cavity. The differences observed in migration of human endometrial stromal cells (hESC) obtained from patients with endometriosis versus healthy controls were proposed to correlate with the abnormal activation of Raf-1/ROCKII signalling pathway.

Objective: To investigate the molecular mechanisms by which Raf-1/ROCKII signaling regulates cytoskeleton remodeling and motility of hESC in the absence of estrogen stimulation.

Materials and methods: To evaluate the mechanism by which Raf-1 regulates cytoskeleton reorganization and motility, we used primary eutopic (Eu-, n=16) and ectopic (Ec-, n=8; isolated from ovarian cysts) hESC of patients with endometriosis and endometriosis-free controls (Co-hESC, n=14). Western blot analyses for Raf-1 and cytoskeleton proteins paxillin, E/R/M, and, MYPT1 and MYPT1as well as their phosphorylaton were performed. Co-mmumoprecipitation assays for MYPT1and Raf-1 were done. Raf-1 siRNA or control siRNA were used to knockdown Raf-1 in hESC. Cell migration assay was performed. The changes in cytoskeleton organization were evaluated by Immunofluorescence analysis of vimentin, phalloidin and Raf-1 in hESC after Raf-1 knockdown.

Results: Raf-1 siRNA knockdown in Co- and Eu-hESC resulted in contraction and decreased migration vs. siRNA controls. This phenotype was reversed following the re-expression of Raf-1 in these cells. Lowest Raf-1 levels in Ec-hESC were associated with hyperactivated ROCKII and ezrin/radixin/moesin (E/R/M), impaired migration and a contracted phenotype similar to Raf-1 knockdown in Co- and Eu-hESC. We further show that the mechanism by which Raf-1 mediates migration in hESC includes direct myosin light chain phosphatase (MYPT1) phosphorylation and regulation of the levels of E/R/M, paxillin, MYPT1 and myosin light chain (MLC) phosphorylation indirectly via the hyperactivation of ROCKII kinase.

Conclusion: Our findings suggest that cellular levels of Raf-1 adjust the threshold of hESC migration in endometriosis.

T-078-2

The microRNA miR-145 Modulates Endometriotic Cell Invasiveness and Stem Cell Properties by Targeting Cytoskeletal Elements and Pluripotency Factors. Ludwig Kiesel,[1,2] Marlene Adammek,[1,2] Burkhard Greve,[1,2] Nadja Kassens,[1,2] Anna Starzinski-Powitz,[1,2] Martin Gotte.[1,2] [1]*Departments of Gynecology and Obstetrics, Radiotherapy and Radiation Biology, Muenster University Hospital, Muenster, Germany;* [2]*Department of Biology, University of Frankfurt, Germany.*

Introduction: microRNAs are small noncoding RNAs which regulate gene expression at the posttranscriptional level (Götte 2010). Dysregulated expression of microRNAs in endometriosis has been independently demonstrated by several investigators, however, functional studies are scarce. The objective of the present study was to identify and confirm target genes and proteins of miR-145, previously shown to be misexpressed in endometriotic tissue (Ohlsson Teague et al. 2009), and to study the functional consequences of miR-145 dysregulation using an *in vitro* system.

Methods: The endometriotic cell line 12Z (Zeitvogel et al. 2001) was transiently transfected with pre-miR-145 precursors, control miRNA or anti-miR-145 (Götte et al. 2010). Predicted miR-145 target gene expression, as identified by screening of the DIANA and microRNA.org databases was monitored by qPCR or Western blotting. miRNA-dependent changes in cell proliferation and invasiveness were evaluated by MTT assay and matrigel invasion assays, respectively. Regulation of the 3'UTR of JAM-A was investigated by luciferase activation assays. Stem cell properties were investigated by ALDH activity assays and flow cytometric side population analysis.

Results: Ectopic expression of miR-145 induced a significant 34% reduction in 12Z cell proliferation and a significant 80% inhibition of matrigel chamber invasiveness ($p<0.05$). qPCR revealed a significant downregulation of mRNA expression for the cell adhesion molecule JAM-A, and the cytoskeletal elements fascin and PODXL by about 70%. Expression of the pluripotency factors SOX2, KLF4, and OCT4, recently found to be dysregulated in endometriosis (Götte et al. 2011) was downregulated by about 50% upon ectopic miR-145 expression ($p<0.01$). Flow cytometric investigations revealed a decrease in the fraction of side population cells and of ALDH-positive cells upon ectopic miR-145 expression. In contrast, ACTG2 was upregulated about 8-fold in miR-145 overexpressing cells ($p<0.05$). Downregulation of JAM-A and fascin was confirmed at the protein level by Western blotting. In addition, direct miR-145-dependent downregulation of JAM-A via its 3'UTR was confirmed using luciferase assays.

Conclusion: miR-145 modulates endometriotic cell proliferation and invasiveness by targeting the expression of cell adhesion molecules, cytoskeletal elements and pluripotency factors. Ectopic expression of miR-145 may emerge as a novel future therapeutic concept in endometriosis.

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T-079

Abnormal Folate Metabolism and the Development of Transposition of the Great Arteries. Ray O Bahado-Singh, Rashmi Bolinjkar, Rita Zafra, Samet Albayrak, Michael Kruger. *School of Medicine, Wayne State University, Detroit, MI, USA.*

Background and Objective: Animal, clinical and epidemiologic data indicate that abnormal maternal or fetal folate metabolism is a risk factor for fetal CHD. This risk reportedly is highest for conotruncal defects. We evaluated whether newborn blood methionine levels (MET), a marker of folate sufficiency correlated with risk of transposition of the great artery (TGA), a common conotruncal defect.

Study Design: Methionine (MET) levels were obtained as part of the Michigan Department of Community Health newborn screening program. Tandem mass spectrometry was used to measure blood MET concentrations. MET levels were obtained at two days of age and therefore reflected fetal levels. We compared 116 cases of TGA and matched 116 normal controls. Factors potentially affecting MET levels namely newborn gender, twin/singleton studies, birth weight (BW), age at testing, blood transfusion, total parenteral nutrition, CHD status, and maternal and paternal race, were examined using univariate analysis to determine they correlated with MET levels. Forced entry and stepwise forward logistic regression analyses were used to identify factors including, (MET levels) that significantly correlated with TGA status. OR was calculated and $p<0.05$ defined significance.

Results: Mean (SD) GA at delivery was not significantly different case versus controls: 38.5 (1.7) vs 38.5 (1.7) weeks ($p=0.9$) while BW was: 3141 (585)g vs 3357.5 (575.3)g ($p=0.005$) and Met 35.8 (27.9) vs 31.8 (8.4) ng/dL ($p=0.15$) . BW had a negative correlation with TGA status ($p = 0.006$) on univariate analysis while MET ($p = 0.9$) and the other factors evaluated did not. There was a significant correlation ($p=0.001$) between BW and TGA on both forced entry ($p= 0.001$) and stepwise forward logistic regression analyses ($p = 0.003$) but not for MET levels and TGA ($p = 0.29$ and $p = 0.36$). No other factor correlated significantly with newborn TGA status.

Conclusion: Using a large number of non-syndromic TGA cases, we were not able to identify significant evidence of fetal folate metabolic dysfunction, as reflected by newborn MET levels, in TGA. The finding appears to contradict other published data regarding the association between folate abnormalities. Other markers of folate metabolism need to be investigated.

T-080

Uterine Artery Doppler, Placental Volume and the Risk for Birth Weight ≤ the 5th Percentile. Nicholas J Behrendt,[1] Darleen Cioffi-Ragan,[1] Leslie Meyers,[1] Anne Mailhot,[1] Nancy West,[2] John C Hobbins,[1] Lorraine Dugoff.[1] *[1]Maternal Fetal Medicine, University of Colorado School of Medicine, Aurora, CO, USA; [2]Epidemiology, University of Colorado School of Medicine, Aurora, CO, USA.*

OBJECTIVE:

Infants with birth weight ≤ 5th percentile (BWt≤5) have greater morbidity and mortality compared to larger infants. Identifying pregnancies at risk for BWt≤5 could lead to early interventions to reduce the morbidity and mortality of this complication. Our objective was to evaluate the risk for BWt≤5 by studying first trimester placental volume quotients (PQ) and uterine artery (UtA) scores.

STUDY DESIGN:

This is a prospective cohort study of 522 women presenting for first trimester aneuploidy screening. Women between 10 3/7 and 13 6/7 weeks gestation had three-dimensional placental volumes and UtA Doppler velocimetries performed transabdominally with a Voluson E8 (GE Healthcare, Milwaukee, WI). Resistive Index (RI), uterine artery notching, and placental volume were assessed. The PQ was calculated by dividing the placental volume by crown rump length. The UtA score was calculated by assigning one point for an RI >0.70 (>75th percentile) and one point for a diastolic notch. A UtA score was calculated for each artery with points combined for a total of 0-4. Logistic regression was used to investigate the associations of PQ and UtA score with BWt≤5.

RESULTS:

The PQ was not significantly associated with BWt≤5 (P=.50). There was a three-fold increase in BWt≤5 for gestational age in patients with UtA scores of 3 or 4 in comparison to scores of 0, 1, or 2 (OR= 3.0, 95% CI: 1.01-8.83, p<0.05). This relationship remained significant after adjusting for age, BMI, parity, PAPP-A level and PQ.

CONCLUSION:

Placental volume does not appear to be associated with BWt≤5. A combination of increased RI and notching in the first trimester (UtA score of 3 or 4) is associated with BWt≤5. First trimester UtA score may potentially be used to identify patients at increased risk for BWt≤5 who may benefit from early prophylactic intervention and increased surveillance. Additional larger studies are warranted to confirm this finding and identify other predictors of BWt≤5.

T-081

First Trimester Homocysteine, Folate, sFlt-1 and PlGF Levels Are Associated with Fetal Growth. Nienke E Bergen,[1,2] Vincent W Jaddoe,[1,3,4] Jan Lindemans,[5] Henk Russcher,[5] Albert Hofman,[4] Eric A Steegers.[2] *[1]The Generation R Study Group, Erasmus University Medical Center, Netherlands; [2]Obstetrics and Gynecology, Erasmus University Medical Center, Netherlands; [3]Pediatrics, Erasmus University Medical Center, Netherlands; [4]Epidemiology, Erasmus University Medical Center, Netherlands; [5]Clinical Chemistry, Erasmus University Medical Center, Netherlands.*

Background Fetal growth restriction is a major determinant of neonatal morbidity and mortality. Optimal placental function is of main importance for the growth and development of the fetus. First trimester homocysteine (tHcy) and folate levels, but also angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) have been suggested to affect fetal growth. However not much is known about the effect of these biomarkers on various fetal growth characteristics in different periods of pregnancy.

Objective To investigate the associations of first trimester tHcy, folate, sFlt-1 and PlGF levels with fetal growth characteristics repeatedly measured in pregnancy.

Methods This study was performed in 5813 women, participating in a population-based prospective cohort study from fetal life onwards. Blood was drawn in early pregnancy (median 13.2 weeks) to analyze tHcy, folate, sFlt-1 and PlGF levels. Fetal growth characteristics were measured in the second and third trimester of pregnancy by ultrasound. Information on birth weight, birth length and head circumference was retrieved from medical records. Multivariate analyses were used.

Results We observed a significantly decreased growth rate of estimated fetal weight and head circumference in women with tHcy levels above the 80th percentile and folate, sFlt-1 and PlGF levels below the 20th percentile relative to the reference group (women with tHcy levels below the 20th percentile and folate, sFlt-1 and PlGF levels above the 80th percentile). Women with lower folate and sFlt-1 levels had also a significantly reduced fetal length growth. Cross-sectional analyses showed that the effect of higher tHcy and lower folate, sFlt-1 and PlGF levels becomes apparent from the third trimester onwards.

Conclusion Our results suggest that higher tHcy and lower folate, sFlt and PlGF levels are associated with a significantly reduced growth rate from the third trimester onwards. Future studies are warranted to determine possible consequences for postnatal growth and development.

T-082

Influence of Maternal Age on Birth Outcomes in Smoking Mothers. Ira M Bernstein,[1] Recia Frenn,[1] Gary J Badger,[2] Sarah Heil,[3] Stephen Higgins.[3] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA; [2]Medical Biostatistics, University of Vermont; [3]Psychiatry, University of Vermont.*

BACKGROUND: Maternal cigarette smoking during pregnancy is associated with reduced fetal growth and an increase in preterm birth. We sought to determine the independent effect of adolescent maternal age on these outcomes in a group of smoking mothers.

METHODS: One hundred and seventy two pregnant smoking women with singleton gestations were evaluated who had been involved in a randomized prospective study examining the effect of redeemable vouchers contingent on evidence of smoking abstinence. We examined birth weight as well as gestational age at delivery comparing mothers who were ≤ 19 years of age at study enrollment (n=34) with older mothers. We performed stepwise regression analysis controlling for smoking volume, parity, infant sex, body mass index (BMI), socioeconomic status, illicit drug use, randomized study group allocation and co-morbid maternal health conditions. P<0.05 accepted for significance.

RESULTS: Maternal age ≤ 19 was associated with a 1 week extension of pregnancy when controlled for co-variables. (age ≤ 19: 39.6 ± 1.7, age ≥ 20: 38.6 ± 2.0 weeks gestation, mean ± s.d., p=0.02). Newborn size was significantly and positively associated with gestational age (r^2=0.47, p<0.0001), maternal BMI (r^2=0.03, p=0.003), male infant sex (r^2=0.023, p= 0.004) and pregestational diabetes (r^2=0.023, p=0.005) and negatively associated with nulliparity (r^2=0.03, p=0.002), and smoking volume (r^2=0.03, p<0.0001). We found no independent association of newborn size with maternal adolescent age.

CONCLUSIONS: In smoking mothers adolescent age is independently associated with prolonged gestational age at delivery and has no independent effect on newborn size when confounding variables are accounted for.

T-083

How Accurate Is Ultrasound in Predicting Birth Weight Discordance in Twin Gestations? Brianne Bimson, Rachelle A Schwartz, Mary Morrissey, Benjamin Lust, Lois Brustman. *Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, New York, NY, USA.*

Objective: To determine the accuracy of ultrasound predicted inter-twin-discordance.

Methods: All twins between 1/2006-7/2011 were reviewed. Inclusion criteria included: documented birth weights, gestational age ≥24 weeks at delivery, and ultrasound estimated fetal weight (EFW) within 7 days of delivery. Percent discordance was calculated using the ultrasound EFW's (predicted discordance) and the birth weights (actual discordance). The absolute discordance error between the actual and predicted discordance was ascertained for each twin set. The absolute error and absolute percent error between the EFW and actual birth weight were also calculated. Statistical analysis was performed using paired t-test.

Results: 241 twins were included. Mean gestational age at birth was 35.8 +/- 2.4 weeks. Mean days between ultrasound and delivery was 3+/-2days. The majority (80%) of twins were dichorionic. There were no differences between absolute error or absolute percent error when comparing twin A to B (191+/-206g vs 196+/-170g, p=0.23 and 8.1+/-8.3% vs 9.0+/-8.6%, p=0.24, respectively). Mean actual discordance was 11.1 +/-9.2% vs mean predicted discordance 9.5+/-9.1% (p<0.01). Forty three (18%) patients had ≥20% discordance at birth. The majority of these twin sets, 72% (31/43), had ultrasound predicted discordances that were less than the actual.

Conclusion: Ultrasound performed near delivery, is an acceptable predictor of birth weight in twins, however the majority of twins with a clinically significant discordance (≥20% at birth) where not identified by sonogram.

Thursday

T-084

The Carbohydrate-Rich Dietary Pattern Used during the Periconceptional Period Is Associated with First Trimester Growth: The Generation R Study.
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Objective: First trimester growth impairment has been associated with pregnancy complications and adverse birth outcome. Knowledge on possible influences of specific maternal dietary patterns on embryonic growth is lacking. This study aims to identify periconceptional maternal dietary patterns in association with first trimester growth and to study the subsequent effects on fetal growth and birth weight.
Methods: From a population-based prospective birth cohort study in the <st2:place w:st="on"><st2:country-region w:st="on">Netherlands</st2:country-region></st2:place>, 847 pregnant women were eligible for this study. Information on nutritional intake was collected by a semi-quantitative food frequency questionnaire. For extracting dietary patterns from this data, Principal Component Factor Analysis (PCA) was used. Outcomes comprised of first trimester growth using the crown-to-rump length (CRL) and fetal growth using estimated fetal weight (EFW) in the second and third trimester, and birth weight.
Results: A 'Carbohydrate-rich dietary pattern' was identified using PCA, characterized by high intakes of bread, margarine, nuts, sweets, processed meat products and non-sweetened, non-alcoholic beverages. After adjustment for confounders a significant association between intermediate (difference, mm: 1.28, 95% CI 0.18;2.38) and high adherence (difference, mm: 1.24, 95% CI 0.14;2.35) to this dietary pattern and CRL (p-trend 0.021) was shown. Increasing adherence to the Carbohydrate-rich dietary pattern was associated with EFW in the second trimester (p-trend 0.029), but neither with EFW in the third trimester nor with birth weight.
Conclusion: This study suggests that increasing adherence to a Carbohydrate-rich dietary pattern is significantly associated with increased growth in the first and second trimester. The clinical relevance of these findings needs further investigation.

T-085

***In Vitro* Human Placental Studies To Support an Adenovirus-Mediated VEGF-D$^{\Delta N\Delta C}$ Gene Therapy Treatment of Severe Fetal Growth Restriction.**
Michelle Desforges,[1] Anna L David,[2] Susan L Greenwood,[1] Colin P Sibley,[1] Paul Brownbill.[1] *[1]Maternal & Fetal Health Research Centre, University of Manchester, United Kingdom; [2]Institute for Women's Health, UCL, United Kingdom.*
Introduction: Impaired utero-placental blood flow leads to severe fetal growth restriction (SFGR). Transduction of pregnant sheep uterine arteries mid-gestation with Ad.VEGF-D$^{\Delta N\Delta C}$ (Ark Therapeutics) reduces their contractile response long term and leads to adventitial neovascularisation, suggesting this might therapeutically prolong fetal growth *in utero* in human pregnancies complicated by SFGR.
Aim: To study adverse structural and functional toxic effects of Ad.VEGF-D$^{\Delta N\Delta C}$ on the human placenta.
Methods: *In vitro* human villous explants from normal term placentas (n=6; which generate a new syncytiotrophoblast, STB, layer in culture) were cultured for 7 days and treated on day 5 for one hour with Ad.VEGF-D$^{\Delta N\Delta C}$ ($5x10^{7-10}$ virus particles / mL), Ad.LacZ ($5x10^{10}$ vp / mL) or virus formulation buffer (FB). Thereafter medium was assayed for hCG release (endocrine homeostasis) and LDH (cell death), until day 7 when fragments were fixed for beta-galactosidase immuno- (β-gal; Ad.LacZ tissue accessibility) and H&E staining. Intact placental lobules were dually perfused *ex vivo* in open-circuit from normal pregnancies and treated with Ad.VEGF-D$^{\Delta N\Delta C}$ (n=3; $6.7e^{11}$ Ad.VEGF-D$^{\Delta N\Delta C}$ viral particles), or FB control (n=2); administered into the intervillous space (IVS), followed by 5 minute perfusion stasis. Perfusion resistance, paracellular permeability, STB shedding, hCG and LDH release were assessed.
Results: Viral treatment caused no detectable effect on tissue structural integrity. Viral vector access was confined to limited areas of the new STB layer with occasional staining of cells in the villous stroma. Explant hCG secretion and LDH release in Ad.VEGF-D$^{\Delta N\Delta C,}$ Ad.LacZ and FB treated groups was similar (Kruskal Wallis). Preliminary data suggest that there was no detectable toxic effect of Ad.VEGF-D$^{\Delta N\Delta C}$ on any placental perfusion indices studied.
Conclusion: Ad.VEGF-D$^{\Delta N\Delta C}$ *in vitro* has no effect on human placental explant tissue structural integrity, cell survival, or hormone secretion, despite evidence that the virus can gain limited access to the STB. Preliminary data also do not indicate any toxic effect of Ad.VEGF-D$^{\Delta N\Delta C}$ on perfusion indices. These data suggest that this potential gene therapy approach has no toxic effects on the placenta.

T-086

Detection of Small for Gestational Age Fetuses Using Customized Standards To Calculate Estimated Fetal Weight in Twin Gestations.
Carlos A Carreno, JM Mastrobattista, Benjamin A Kase, Brendan D Connealy, Sean C Blackwell. *Obstetrics, Gynecology and Reproductive Sciences, University of Texas at Houston, Houston, TX, USA.*
Objective: Customized birth weight standards have been shown to be better associated with adverse pregnancy outcomes compared to population-based standards. We intend to determine rate of small for gestational age (SGA) infants in twin gestations using customized estimated fetal weights (custEFW) versus estimated fetal weight (EFW) calculated using ultrasound and growth percentiles determined using population (popEFW) growth curves.
Methods: Historical cohort of women with twin gestation delivered at our institution from July 2010 till July 2011. Congenital and chromosomal anomalies as well as monoamniotic twins were excluded from the study. Ultrasound-derived EFW and growth percentiles (popEFW) were reported utilizing the Hadlock formula and the percentile table devised by Williams et al. (Obstet Gynecol 1982. 59:624-32). A customized EFW (custEFW) was calculated adjusting for maternal height, weight, ethnicity, parity, and fetal sex (www.Gestation.net). Rates of SGA (birthweight < 10%ile) were compared between the study groups.
Results: A total of 82 women (75 dichorionic and 7 monochorionic twin gestations) met inclusion criteria. popEFW identified 5 cases of SGA in twin A versus 28 SGA cases that were identified using custEFW. There were 3 cases of SGA in twin A identified by popEFW and cust EFW. In twin B there were 21 cases of SGA using custEFW and 18 SGA cases were identified by custEFW that were missed by popEFW.
CONCLUSION: This is the first study to date using custEFW centiles for prenatal ultrasound diagnosis of abnormal fetal growth in twin gestations. Using custEFW centiles allowed us to detect higher rates of SGA fetuses in our twin gestation population.

T-087

Effect of Glucocorticoids in an In-Vitro Model of Neuroal Cell under Hypoxic Conditions.
Carlos A Carreno,[1] Joseph L Alcorn,[2] Manju Monga,[1] Susan M Ramin,[1] Karen Bishop,[1] Alex C Vidaeff.[1] *[1]OBGYN, University of Texas Houston, Houston, TX, USA; [2]Pediatrics, University of Texas Houston, Houston, TX, USA.*
Objective: Fetal growth restriction is frequently associated with chronic hypoxia. Animal models indicate that exposure to corticosteroids may enhance hypoxic neuronal injury but its effects on the brain of growth-restricted human fetuses remain largely unstudied. We investigated if corticosteroid exposure contributes to neuronal injury in conditions of hypoxia in an in vitro model of differentiated human neurons.
Methods: Human teratocarcinoma cells were differentiated into NT2-N neurons, akin to human fetal neurons, after treatment with retinoic acid. Cells were exposed to hypoxic or normoxic conditions for 6 hours and then incubated in the absence or presence of dexamethasone (DXM, 10-7 M) for 48 hours. Neurotoxicity, reflected by apoptosis, was determined using a caspase-3-specific activity assay and western blotting. Functional neuronal impairment was assessed by S-100B protein expression, determined by western blotting. Caspase-3 is a key marker of apoptosis and S-100B is a useful biochemical marker of brain damage. Caspase-3 values were calculated as a percentage of mean caspase-3 cleavage and S-100B values are expressed as arbitrary units. Numeric values are expressed as mean (± SEM). Wilcoxon test was conducted for statistical comparisons.
Results: Both pro-caspase-3 (32 kDa) and cleavage product caspase-3 (17kDa) were expressed in culture. DXM exposure resulted in increased caspase 3-cleaved products compared to control: 28 (±9) vs 3 (±1), p = .01. However, the pro-apoptotic effects of DXM were not significantly intensified by pre-exposure to hypoxia: 38 (±34) vs 28 (±9). A 2-fold increase in S-100B over control was observed after DXM exposure: 88695 (±23559) vs 39302 (±2960),

p = .02. Post-hypoxic DXM exposure did not result in significantly higher S-100B values compared to normoxic conditions.
Conclusion: We demonstrated in vitro that although DXM exerts toxicity on normoxic neurons, prior exposure to hypoxia does not increase neuronal sensitivity to DXM. No additive or synergistic effect was observed when DXM incubation followed oxygen deprivation. These results may be relevant to events hypothesized to occur in the hypoxic brain of premature growth restricted fetuses exposed to corticosteroids.

T-088

Chronic Norepinephrine Exposure Desensitizes Fetal Sheep Islets and Enhances Insulin Secretion. Xiaochuan Chen, Dustin T Yates, Antoni R Macko, Amy C Kelly, Sean W Limesand. *Department of Animal Sciences, University of Arizona, Tucson, AZ, USA.*
Placental insufficiency lowers fetal oxygen and glucose concentrations, which chronically elevate fetal plasma norepinephrine (NE) concentrations. Previous studies in uncompromised sheep fetuses designed to isolate NE actions show that chronic exposure to NE suppresses insulin secretion through α2-adrenergic receptors (ARs) and increases plasma glucose. Removing fetuses from the chronic NE exposure results in acute β-cell hyper-responsiveness to glucose with lower mRNA expression of α2-ARs in pancreatic islets. This study is to determine if the compensatory mechanism of NE is dependent on hyperglycemia and persists in fetal sheep after chronic NE exposure. NE was continuously infused into fetal sheep at 1-4 μg/min through 131-137 days of gestational age. Insulin was infused into the ewe to maintain fetal euglycemia during the NE treatment period. In vivo, fetal glucose stimulated insulin secretion (GSIS) was tested with a square-wave hyperglycemic clamp prior to chronic treatments, 1-day, and 5-day after terminating the infusion. After the last GSIS study, in vitro, islets were isolated and incubated with 11.1 mM glucose and various NE concentrations (0, 0.0001, 0.001, 0.01, 0.1, 1, and 10 μM) to determine insulin release. During the 7-day treatment period, NE-infused fetuses had 10 fold greater ($P<0.05$; n=8) plasma NE concentration than vehicle infused controls (n=7), and plasma insulin concentrations were lower (0.17±0.02 vs 0.43±0.03 ng/ml, $P<0.01$) than controls. Plasma glucose concentrations were not different between treatment groups. GSIS responsiveness before treatment was similar between groups. However, both 1-day and 5-day after discontinuing the infusion, NE fetuses had 2-fold greater ($P<0.05$) insulin concentrations than controls at the hyperglycemic steady state conditions. In static incubations, islets from NE fetuses were less ($P<0.05$) responsiveness to NE inhibition than control islets with the IC50 of 5.40±1.10 vs 2.17±0.90 nM. Islets from NE fetuses also showed greater maximal ($P<0.05$) insulin secretion than controls (2.12±0.20 vs 1.38±0.15 ng/h/islet, $P<0.05$). These findings show that chronic NE exposure, not hyperglycemia, leads to hyper insulin secretion coupled with desensitized α2-ARs signaling. Moreover, this compensatory enhancement in fetal β-cell persists for 5-day after removing NE infusion indicating continuingly β-cell impairment in later life.

T-089

Risk of SGA among Indicated Versus Spontaneous Early Preterm Births. Brendan D Connealy,[1] Benjamin A Kase,[1] Carlos A Carreno,[1] Jerrie S Refuerzo,[1] George Saade,[2] Sean C Blackwell.[1] *[1]Obstetrics, Gynecology, and Reproductive Sciences, University of Texas Health Science Center, Houston, TX, USA; [2]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Objective:
To describe the rate of small for gestational age (SGA) fetuses in women with indicated and spontaneous early preterm birth (PTB) < 34 weeks using customized standards.
Study Design:
All singleton, non-anomalous, liveborn PTBs 20 wks 0 days to 33 wks 6 days at a tertiary care hospital over a one year period (Jan 1-Dec 31, 2010) were studied. Early PTBs < 34 weeks were categorized as spontaneous (PTL or PPROM), medically indicated (MI), or non-medically indicated (NMI). For each pregnancy, customized neonatal birth weight (cust BW) was calculated using maternal height, weight, ethnic group, parity and fetal sex (www.Gestation.net). The frequency of SGA (cust BW< 10th%tile) was compared between PTB subtypes across different GA ranges.
Results:
There were 5,082 total live births of which 342 (6.7%) met inclusion criteria. There were no differences in the median customized birthweight centiles based on PTB subtypes; PTL 19.7 %tile (IQR 4-48.1), PPROM 18.8 %ile (IQR 5.7-50.6 %ile), and MI PTB 24.0 %ile (IQR 2.6-38 %ile) (p=0.057). There was an increase in SGA among women with MI PTB between 24-28 weeks EGA.

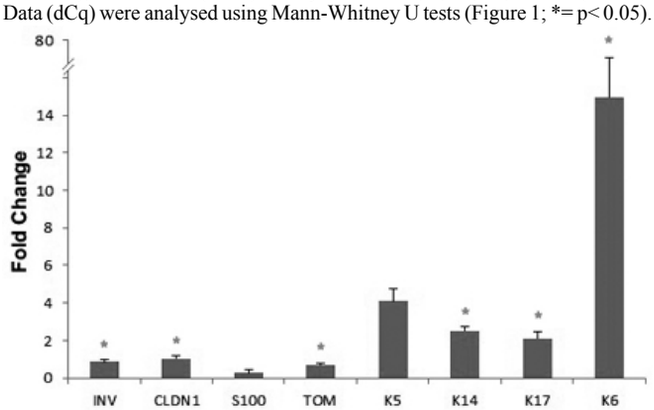
Frequency of SGA by PTB subtype across GA ranges 20-34 wks

	PTB Subtype			
Gestational Age	sPTL	PPROM	MI	P-value
20-24	30.8%	66.7%	50%	0.54
24-28	26.7%	13.3%	73.1%	<0.001
28-32	51.3%	43.8%	40%	0.57
32-34	29.8%	41.7%	40%	0.48
Total	35.3%	34.8%	46.6%	0.120

Conclusions:
Over one-third of PTB's < 34 wks were SGA, but the frequency did not differ based on PTB subtype. MI PTB between 24-28 wks had the highest rate of SGA.

T-090

Antenatal Corticosteroid Administration Induces Selective Maturation of the Fetal Ovine Epidermis. Thomas W Cox,[1] Masatoshi Saito,[2] John P Newnham,[1] Jeffrey A Keelan,[1] Matthew W Kemp.[1] *[1]School of Women's and Infants' Health, University of Western Australia, Perth, Western Australia, Australia; [2]Department of Obstetrics & Gynecology, Tohoku University, Sendai, Miyagi, Japan.*
Objective
Corticosteroid administration to women at risk of preterm delivery reduces the incidence of neonatal death and respiratory disease by functionally maturing the fetal lung. However, the effect(s) of antenatal corticosteroid administration on the development of the fetal skin are poorly understood.
Using an ovine model of preterm birth we aimed to examine whether maternally administered antenatal corticosteroids alters development of the fetal skin.
Methods
Date mated merino ewes were divided into two groups: i) IM 150 mg depo-relovera + 0.5 mg/kg betamethasone (n=7); and ii) IM 2 ml saline (control, n=4). Ewes were anaesthetised and the fetuses surgically delivered 48 hours post intervention at 124d GA (± 2 d; term=150 d). Fetal skin from the inner left thigh was collected for quantitative PCR analysis. The expression of 12 epidermal protein targets was examined: Involucrin (INV), Occludin (OCC), Filaggrin (FIL), Trappin Ovine Molucule (ovine Elafin; TOM), S100A7, Claudin 1 (CLDN1), E-Cadherin (CDH) and cytokeratins 5, 6, 10, 14, and 17 (K5-17). Data (dCq) were analysed using Mann-Whitney U tests (Figure 1; *= p< 0.05).



Results
Relative to control, exposure to betamethasone significantly increased the expression of INV (p= 0.01), TOM (p= 0.01), CLDN1 (p= 0.01), K6 (p= 0.029), K14 (p= 0.016) and K17 (p= 0.01).
Conclusions
These novel data demonstrate that antenatal corticosteroid administration markedly alters the expression of structural proteins in the developing fetal epidermis. These data suggest a selective maturation of the fetal epidermis characterised by precocious development of the CCE, epidermal differentiation (K14), and up regulation of wound-response proteins (K6 and K17). The functional implication(s) of these changes warrant further investigation given the prevalence of cutaneous infection and barrier dysfunction in the preterm population.

T-091

Stillbirth and Pregnancy Resorption in Female Mice Transgenic for Angiotensinogen or Renin. Jeff M Denney, Cynthia E Shaw, Annette Gendron, Dinesh M Shah. *Maternal-Fetal Medicine, Perinatal Research, and Comparative Pathology, University of Wisconsin, Madison, WI, USA.*

Objective: Crossing a female mouse transgenic for human angiotensinogen (hAng) with a male mouse transgenic for human renin (hRen) has been reported as a model for preeclampsia. Previous in vitro studies have indicated no interaction between human RAS transgenes and mouse RAS genes due to species-specific biological actions. Because uterus has an independent RAS, especially in the gravid state, we compared wild type (WT) with transgenic (TG) gravid mice to determine the affect of transgene presence on fetal outcome.

Methods: Mouse genotyping was performed for verification of lines. Colony lines of hAng, hRen, and WT were maintained separately during breeding protocol to avoid hAng x hRen interaction. Progeny from breeding were counted. Necropsy was performed on gestation day 19 of subjects designated for histologic analysis. Uteri and products of conception were resected en bloc, evaluated grossly, and weighed. Univariate analysis was used where appropriate.

Results: 20 pregnancies were analyzed. TG females (hRen or hAng) delivered significantly less live born pups per gestation when compared to WT counterparts. TG females had a significantly higher stillbirth (SB) rate. Mean uterine-conceptus (U-C) weights of transgenic females were smaller compared to those achieved by their wild type counterparts (Table 1). Histologic examination of the uteroplacental interface revealed increased placental necrosis, fibrin deposits, inadequate vascular modeling, and inadequate development of the villous labyrinth in TG females. WT uterine-conceptus blocks were unremarkable.

Table 1: Fetal Outcome by Maternal Genotype

Mean	WT	TG	p-value
LB/gestation	6.6	1.5	0.038
SB/gestation	0	1.5	0.026
U-C (gm)	2.3	0.8	NS

Conclusion: Mice transgenic for either renin or angiotensinogen have lower live birth rates and higher still birth rates even when interactions of the hRen with hAng are avoided in breeding. Given significant effects on live birth rate and a trend toward low uterine-conceptus weight, these data suggest abnormality that may be a consequence of human transgene RAS interactions with native mouse RAS genes. Our observations indicate increased pregnancy resorption and failure to establish viable pregnancy in the transgenic mouse. Our observations also suggest placental hypoxia due to inadequate vasculature modeling. These data suggest interspecies interactions of RAS genes in vivo.

T-092

Maternal Nutrient Restriction (MNR) during Pregnancy Redirects Fetal Baboon Skeletal Muscle (SM) miRNA Expression Profile To Promote a Mesenchymal, Adipose Cell Phenotype. Min Du,[1] Xiu Yan,[1] Mark J Nijland,[2] Thomas J McDonald,[2] Peter W Nathanielsz.[2] *[1]Dept Animal Science, Washington State University, Pullman, WA, USA; [2]OB/GYN, Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*

We have developed a baboon model of moderate MNR that leads to decreased fetal nutrient availability, 15% IUGR and insulin resistant phenotype before puberty. SM is a key tissue responsible for glucose utilization. An individual's full set of SM fibers form in fetal life and there is much interest in the role of miRNA in regulation of development of fetal SM . MNR in pregnant sheep leads to increased SM adipogenesis (J Physiol.;575, 241).

HYPOTHESIS: MNR redirects fetal SM miRNA to support a mesenchymal, adipogenic phenotype.

METHODS: Pregnant baboons ate *ad lib,* controls (CTR) or 70% CTR (MNR) from 0.16 gestation (G) with fetuses recovered at c-section at 0.5 or 0.9 G (3 CTR and 3 MNR each age), fetal soleus SM miRNA analyzed based on all known miRNAs of human and primates (LC Sciences, Houston, TX).

RESULTS: 18 SM miRNAs were differentially expressed at 0.5G and 35 at 0.9G. Of these miRNAs, miR-3960, associated with differentiation, was down-regulated. miRNA including miR-299 miR-495 miR-411, miR-376a and miR-199b which promote cell proliferation and dedifferentiation were up-regulated in MNR. This trend persisted in 0.9G fetal SM, miRNA promoting differentiation including miR-26a miR-119a and miR203 were down-regulated while those promoting proliferation including miR-299 and miR-466 were up-regulated. Most interestingly, the miR200 family which inhibits epithelial-mesenchymal transition (EMT) was undetectable in MNR but expressed in CTR SM; EMT provides cells with stem cell-like dedifferentiated phenotypes.

CONCLUSION: MNR alters fetal SM miRNA profile increasing expression of miRNAs promoting a proliferating, mesenchymal stem cell like phenotype. Changes in miRNA could explain enhanced adipogenesis in MNR offspring SM observed in sheep. HD 21350.

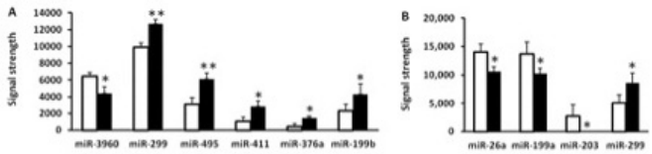


Fig. 1.miRNA expression in CTR (open) and MNR (solid) fetal baboon SM. A) M ± SE 0.5 G; B) M ± SE 0.9 G; (Arbitrary units; *P < 0.05, **P < 0.01; n = 3)

T-093

First Trimester Growth Curves of the Human Embryonic Brain. M Gijtenbeek, I Groenenberg, H Bogers, N Exalto, R Steegers-Theunissen, E Steegers. *Obstetrics & Gynecology, Erasmus MC.*

Background

Recent studies show that brain development is influenced by periconceptional maternal exposures. Derangements in the embryonic brain affect brain function in early and later life. This emphasizes the need for reliable reference growth curves of embryonic brain structures.

Objectives

To assess: 1) first trimester growth curves of embryonic brain structures 2) intraobserver reproducibility.

Methods

From 113 normal singleton pregnancies, we obtained vaginal three-dimensional ultrasonography scans at 7-12 weeks gestation. Where possible, we measured the following brain structures: thickness of diencephalon (DT), mesencephalon (MT), and telencephalon (TT) and total diameter of the diencephalon (DTD) and mesencephalon (MTD). For intraobserver reproducibility, 30 random scans of 30 randomly selected patients were measured twice by one operator. Intraclass correlation coefficients (ICCs) and Bland-Altman plots were used to assess the level of agreement.

Results

Embryonic brain structures could be measured appropriately in 28%, 25% and 23% of the scans, respectively. There was a clear relationship between gestational age and the sizes of the embryonic brain structures ($r^2 > 0.8$)(figure). All ICCs were > 0.99, indicating good reliability. The Bland-Altman plots showed good intraobserver agreement for all measurements (table).

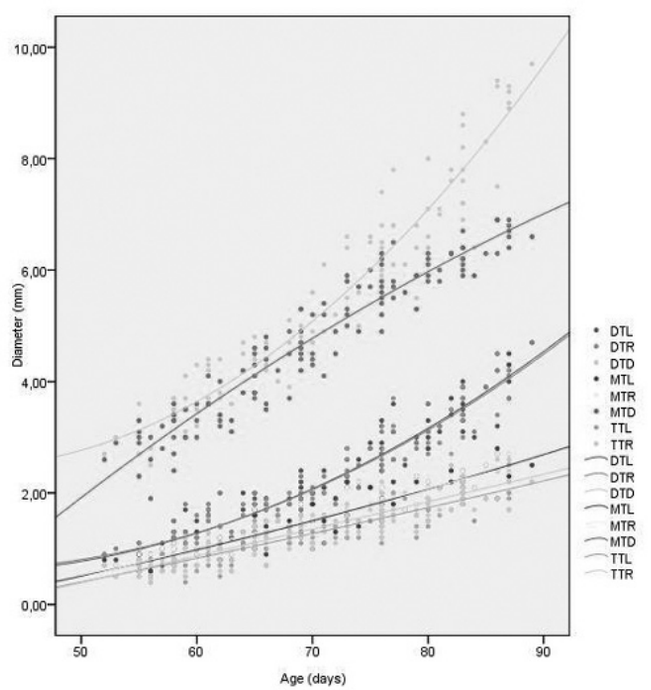


Figure Growth curves of normal embryonic brain structures

Brain structure	Mean % difference	95% limits of agreement
DTL	-0.52	5.45 to -6.48
DTR	-0.72	11.31 to -12.74
DTD	-0.34	2.65 to -3.33
MTL	1.75	13.41 to -9.90
MTR	-0.31	10.61 to -11.23
MTD	0.00	4.46 to -4.45
TTL	-1.82	14.82 to -18.45
TTR	0.29	18.09 to -17.51

Table Intraobserver agreement of the measurements of the embryonic brain structures

Conclusions

It was feasible to create reference growth curves of embryonic brain structures with a very high intraobserver reproducibility. In future, larger datasets will be used for diagnosis and research of abnormal embryonic brain development.

T-094

Molecular Mechanisms Regulating IGFBP-1 Phosphorylation. Maxim Seferovic,[1,2,3] Tony Shen,[1,3] Majida Abushehab,[3] Zarique Akanda,[3] Laszlo Gyenis,[2] David Litchfield,[2] Thomas Jansson,[4] Madhulika Gupta.[1,2,3] *[1]Dept Ped, U of Western Ontario, London, ON, Canada; [2]Biochem, UWO, London, ON, Canada; [3]Children's Health Research Institute, UWO, London, ON, Canada; [4]Center for Pregnancy & Newborn Research, Dept OB/GYN, U of Texas Health Science Center, San Antonio, TX, USA.*

Insulin-like growth factor-I (IGF-I) is a critical regulator of fetal growth, and IGF-I bioavailability is modulated by binding to IGFBP-1, mainly produced by the fetal liver. Phosphorylation of IGFBP-1 markedly increases its affinity to bind IGF-I and IGFBP-1 hyperphosphorylation may constitute a key mechanism by which growth is reduced in IUGR. However, the molecular mechanisms regulating IGFBP-1 phosphorylation are largely unknown. We hypothesized that inhibition of mammalian target of rapamycin (mTOR) signaling and activation of protein kinase CK2 increase IGFBP-1 secretion and phosphorylation. **Methods:** Human HepG2 cells, an established model of fetal hepatocytes, were incubated with the mTOR inhibitor rapamycin (100 nM) and/or a selective CK2 inhibitor, 4,5,6,7-tetrabromobenzotriazole (TBB). Alternatively, cells were transfected with siRNA targeting CK2 (α, α, and β subunits) or scrambled siRNA (control). IGFBP-1 secretion and phosphorylation were determined in conditioned media (CM) using Western blotting. Endogenous CK2 activity was determined using the substrate peptide DSD. **Results:** Rapamycin increased IGFBP-1 secretion ~2.5-fold (n=3; $p<0.05$) and IGFBP-1 phosphorylation. In addition, the affinity of IGFBP-1 for IGF-I increased ~57-fold (KD with rapamycin 2.32×10^{-4} vs. without 4.12×10^{-6}, n=3) in response to rapamycin. HepG2 cells incubated with TBB, showed a time- and dose-dependent inhibition of endogenous CK2 activity. Rapamycin stimulated CK2 by 69% ($p<0.01$, n=3). TBB significantly decreased phosphorylation of IGFBP-1 in CM. Moreover, inhibition of CK2 activity in cells strongly attenuated hyperphosphorylation induced by mTOR inhibition. The CK2-mediated phosphorylation of IGFBP-1 was further confirmed by CK2α silencing. **Conclusion** mTOR inhibition induces marked IGFBP-1 phosphorylation mediated by protein kinase CK2, providing a novel mechanistic link between mTOR and IGF-I signaling. Hypoxia and reduced amino acid concentrations are common in IUGR and well established to inhibit mTOR signaling. We therefore speculate that these mechanisms may contribute to IGFBP-1 hyperphosphorylation, decreased IGF-I bioavailability and reduced fetal growth *in vivo.*

T-095

Use of Individualized Growth Curves To Determine Optimal Growth in Fetuses with Gastroschisis. Bethany L Hart, Stephanie E Mann. *Gynecology and Obstetrics, University at Buffalo.*

OBJECTIVE: Fetal gastroschisis is often complicated by abnormalities in growth; however, prenatal assessment of weight using standard sonographic measurements has been found to be inaccurate. This inaccuracy occurs secondary to standard measurement parameters based on abdominal wall circumference, which is altered in gastroschisis. The purpose of this study is to evaluate the use of individualized fetal growth curves to increase accuracy in predicting neonatal birth weight.

METHODS: This retrospective study included all fetuses diagnosed with gastroschisis and delivered at our regional perinatal center between 2000-10. Incomplete charts or fetuses with other conditions or abnormal karyotypes were excluded. Data collected included: biparietal diameter, abdominal circumference, femur length, estimated fetal weight, gestational age at time of sonogram and delivery, birth weight. Individualized best fit linear curves were generated for each fetus and the % error was calculated as follows: (birthweight - predicted birthweight)/birthweight x 100. Pearson Correlation coeffecients were calculated with $P < 0.05$ accepted as significant.

RESULTS: Complete data were available for 27 fetuses. There is a 0.81 correlation between actual and predicted neonatal birthweight. There was no correlation between the number of ultrasounds performed for each fetus and the absolute % error.

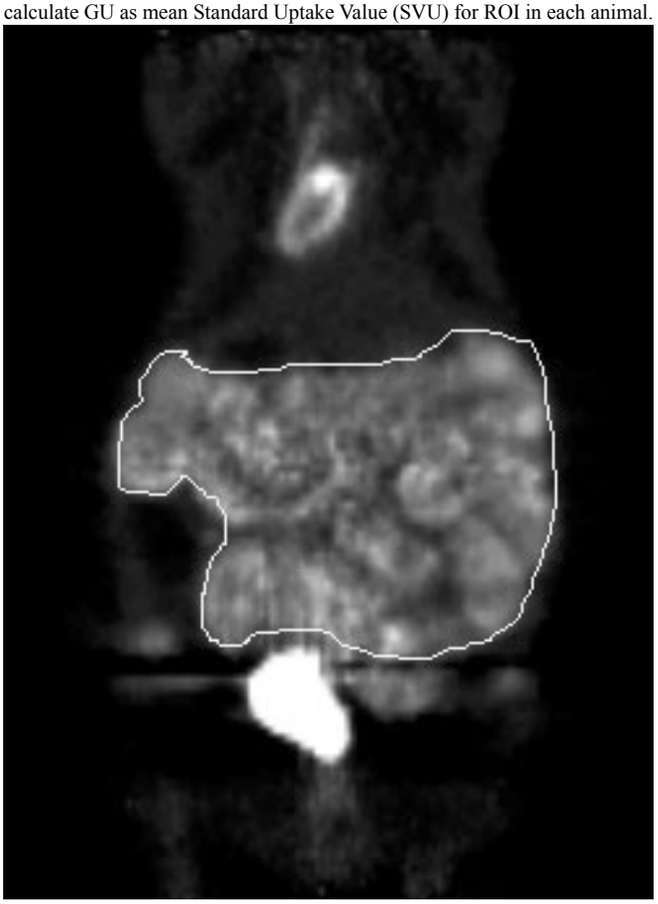
CONCLUSIONS: Accurately estimating fetal weight in gastroschisis is a challenge. Our data suggest that assessing individual fetal growth trajectories may be a more accurate method to evaluate growth in fetuses with gastroschisis.

T-096

MicroPET Imaging in Pregnancy: Decreased Glucose Transport to Placenta & Pup with Maternal Calorie Restriction. Scarlett D Karakash,[1] Hye J Heo,[1] Wade Koba,[2] Fabien Delahaye,[1] Youngmei Zhao,[1] Eugene Fine,[2] Nir Barzilai,[3] Francine H Einstein.[1] *[1]Obstetrics and Gynecology and Women's Health, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, USA; [2]Nuclear Medicine, Albert Einstein College of Medicine, Bronx, NY, USA; [3]Medicine/Endocrinology/Genetics, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, USA.*

Objective: Micro Positron Emission Tomography (mPET), a validated mode of molecular imaging, is used to quantify *in vivo* tissue uptake of glucose using radiolabeled tracers. Our goal was to determine if mPET could be used to measure placenta-pup glucose uptake (GU) in pregnant dams.

Methods: SD dams (n=5/gp) were fed either standard chow *ad libitum*(Con) or calorie restricted (pair-fed 50% kcal/d of Con)(CR) from 11 day of gestation. On day 19-20 of gestation, dams were given intravenous ^{18}F-fluorodeoxyglucose and mPET was performed under inhaled anesthesia. ASIPRO (Siemens) software was used to localize Regions of Interest (ROI), defined as 5 image slices representing the maximum placenta-pup concentration area, and to calculate GU as mean Standard Uptake Value (SVU) for ROI in each animal.



Results: At mPET imaging, Con weighed more than CR (302±8 vs 276±7.5 g, $p<0.01$). Although litter size was similar (Con 12.2±1.3 v CR 11.4±3.2, p=NS), pup weight was greater in CR (Con 2.9±0.5 v CR 3.7±0.3 g, $p<0.05$). Despite this, placenta-pup GU was lower in CR compared to Con (1.7±0.4 v 2.8±0.3 SVU, $p<0.01$).

Glucose Uptake

Rat	Con (Mean SUV)	CR (Mean SUV)
1	3.20	1.87
2	2.90	1.84
3	2.61	2.01
4	2.57	1.78
5	2.57	0.99
Mean	2.77	1.70*
Standard Deviation	0.28	0.41

* p<0.01 complared to control

Conclusions: MPET is a novel tool that may be used in pregnant models to non-invasively quantify *in vivo* glucose transport. Here we demonstrate differential placenta-pup glucose uptake associated with alterations in maternal diet. Future applications may include longitudinal studies and use of other radiolabeled substrates, e.g. amino acids and fatty acids.

T-097

An Insulin Resistant Phenotype in Men Who Father Pregnancies Affected by Fetal Growth Restriction. Sara Hillman,[1] Melissa Whitten,[2] Donald Peebles,[1] David Williams.[2] *[1]Institute for Women's Health, University College London, London, United Kingdom; [2]Insitute for Women's Health, University College London Hospital, London, United Kingdom.*

Background:

Low birth weight is associated with an increased risk of developing type-2 diabetes in later life. The fathers of growth restricted offspring are themselves more likely to develop type-2 diabetes in later life. As part of a larger study to determine whether offspring with fetal growth restriction (FGR) inherit insulin resistance (IR) from their fathers, we designed a case-control study to compare IR and endothelial function in men who father FGR offspring (cases) with men who father normal birth weight offspring (controls).

Methods:

We recruited 42 fathers of on-going FGR pregnancies, (fetal growth <10th customised centile (1.5th centile; 0-5.4th) and confirmed once the pregnancy was completed. We excluded pregnancies with chromosomal or structural abnormalities or maternal diseases. Fathers of normal grown offspring, 10th to 95th centile (49.8th centile; 10.2-95th), were controls (n=73). In all fathers, we measured insulin resistance (HOMA index, from fasting serum glucose and insulin levels), endothelial function by flow-mediated dilatation (FMD), waist-circumference and blood pressure. The data was analysed using multivariate logistical regression (STATA 10 package).

Result

Fathers of FGR pregnancies had higher systolic (p=0.015) and diastolic (p=0.0073) blood pressures and greater waist circumferences (p=0.035) than fathers of normal weight offspring. For every 1% change in paternal insulin resistance the OR of fathering FGR off-spring was 7.5 fold higher (95% CI; 1.15-48.12 p= 0.035) after adjusting for paternal age, smoking and ethnicity. For every 1% increase in FMD men were 17% less likely to father an FGR baby (OR 0.83; 0.72-0.96; p=0.013), but this difference was lost after adjustment for paternal smoking. Men who smoke were 3 times more likely to father an FGR baby (OR 3.00; 1.01-8.90; p=0.047) after adjustment. There were no statistical differences between phenotype of mothers in the two groups.

Conclusions:

Fathers of FGR pregnancies have a metabolic and vascular phenotype present at the time of an affected pregnancy, predisposing them to type 2 diabetes. We plan to investigate whether this observation can be explained by epigenetic modifications in the paternal genome that may be inherited by the growth-restricted fetus.

T-098

Remote Control of Fetal Metabolism by Placental mTOR Signaling. Thomas Jansson,[1] Lena Eliasson,[2] Fredrick Rosario,[1] Theresa L Powell,[1] Madhulika B Gupta.[3] *[1]Center for Pregnancy and Newborn Research, Dept OB/GYN, University of Texas Health Science Center San Antonio, TX, USA; [2]Diabetes Centre, Malmö, Lund University, Sweden; [3]Dept of Pediatrics and Biochemistry, University of Western Ontario, London, ON, Canada.*

Introduction: Placental mammalian target of rapamycin (mTOR) signaling responds to changes in nutrient availability. Inhibition of the TOR signaling pathway in the fat body of Drosophila influences whole body growth mediated by the release of a humoral factor that regulates the secretion of insulin like peptides from the brain (Colombani et al. Cell 2003, 114:739-749). In analogy to the role of TOR in the fat body of Drosophila, we hypothesized that human placental mTOR signaling regulates the release of a circulating factor that alters the phosphorylation and secretion of IGFBP-1 and IGF-I from the fetal liver and the secretion of insulin from fetal beta cells. **Methods**: After 18 hours of culture, primary human trophoblast cells were transfected with (1) scrambled siRNA, (2) siRNA targeting *raptor* (inhibits mTORC1 signaling) or (3) *rictor* siRNA (causing mTORC2 inhibition). Human HepG2 cells (an established model for fetal hepatocytes) were incubated for 24 hours in conditioned media (CM) from control or mTOR-silenced trophoblast cells. Subsequently, we determined IGFBP-1 and IGF-I secretion and IGFBP-1 phosphorylation in HepG2 cell media using ELISAs, and 1-D and 2-D Western blotting. Basal and glucose induced insulin secretion was determined in the rat beta cell line INS 832/13 following a 48-hour incubation in trophoblast CM. **Results**: Incubation of HepG2 cells in CM of *raptor* or *rictor* silenced trophoblast cells resulted in increased IGFBP-1 and decreased IGF-I secretion (p<0.05, n=12). As a result, CM from *raptor* or *rictor* silenced trophoblast cells decreased the ratio of IGF-I and IGFBP-1 secreted from HepG2 cells by more than 50% (p<0.05, n=12). CM from *rictor* silenced trophoblast cells caused a marked hyperphosphorylation of IGFBP-1 (n=3), which is known to increase the binding affinity for IGF-I. Collectively, these changes are expected to decrease IGF-I bioavailability. CM from *raptor* silenced trophoblast cells increased glucose-stimulated insulin secretion from INS 832/13 cells (+66%, n=3, p<0.05). **Conclusion**: These results are consistent with the possibility that placental mTOR signaling regulates the release of circulating factor(s) that influence fetal metabolism and growth.

T-099

Molecular Characterization of In Utero Fetal Lung Injury: The Birth of Bronchopulmonary Dysplasia. Ryan McAdams,[1] Jeroen Vanderhoeven,[2] Richard Beyer,[3] Theo Bammler,[3] Federico Farin,[3] Michael Gravett,[2] Craig Rubens,[4] Kristina Adams Waldorf.[2] *[1]Pediatrics, University of Washington, Seattle, WA, USA; [2]Obstetrics & Gynecology, University of Washington, Seattle, WA, USA; [3]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA; [4]Global Alliance to Prevent Prematurity & Stillbirth, , Seattle, WA, USA.*

Background: Intrauterine exposure to amniotic fluid (AF) cytokines is thought to play a role in development of bronchopulmonary dysplasia (BPD). Our objective was to determine for the first time early molecular pathways associated with fetal lung injury in the saccular stage of lung development, which is when BPD is thought to originate.

Methods: Ten chronically catheterized pregnant monkeys (*Macaca nemestrina*) at 118-125 days gestation (term=172 days) received choriodecidual inoculation of either: 1) Group B Streptococcus 1 x 10^6 colony forming units (n=5) or 2) saline (n=5). Cesarean section and fetal necropsy was performed 4 days after GBS or 7 days after saline infusion to collect tissues. RNA was extracted from fetal lungs and profiled by microarray. Statistical analysis focused on single genes and gene set analysis. Results were validated by RT-PCR (chorioamnion) and Luminex (AF).

Results: Only two GBS animals developed early labor and chorioamnionitis with no cervical change in remaining animals. Despite uterine quiescence in most cases, fetal lung injury occurred in four GBS animals (intra-alveolar neutrophils, interstitial thickening) and was absent in controls. Significant elevations of AF cytokines (TNF-α, IL-8, IL-1β, IL-6) were detected in GBS versus controls (p<0.05). Lung injury was not directly caused by GBS, because GBS was undetectable (AF, fetal lungs) by culture and PCR. A total of 187 genes were differentially expressed in fetal lungs (p <0.05; fold change >2.0) with a striking upregulation of innate and adaptive immunity (e.g. IL-1β, IL-8, chemotaxis, dendritic cell antigen processing, T cell activation). Pathways critical for angiogenesis (ANGPT1, VEGF regulation), morphogenesis (WNT3), and cell survival (aldo-keto reductase family 1 member B10) were downregulated.

Conclusions: A transient choriodecidual infection may induce fetal lung injury without AF infection, chorioamnionitis or preterm labor. Our results provide a window for the first time into early molecular pathways disrupting fetal lung angiogenesis and morphogenesis before preterm labor occurs.

T-100

Sex- and Diet-Related Alterations in Embryonic Brain Inflammasome. Mark C Alanis,[1] Claudia Umphlet,[2] Satomi Kohno,[1] Jianguo Zhu,[1] Ann-Charlotte Granholm.[2] *[1]Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA; [2]Neurosciences, Medical University of South Carolina, Charleston, SC, USA.*

OBJECTIVE: The gestational period is a critical window during which the developing brain is potentially susceptible to inflammatory diets, such as those high in trans fat.

STUDY DESIGN: Fischer 344 virgin females were randomized to isocaloric experimental (TFA diet; 30% kcal from fat, 15% kcal from trans unsaturated fatty acids [TFA]) or control (AIN-93; 10% kcal from fat, 0% from TFA) prior to and during pregnancy. Cesareans were performed on embryonic day 19, and brains from offspring were collected, frozen, and later homogenized. Ribonucleic acid (RNA) was isolated, and a pilot study was performed using an 84-gene polymerase chain reaction (PCR) array of cytokines, chemokines, and their receptors pertaining to inflammation that identified 10 target genes with ≥ 3-fold difference in gene expression between the 2 diet groups. Absolute quantitative PCR was then performed to further investigate these findings. Two-way ANOVA with two-tailed p-values of .05 was used to evaluate the relationships between offspring sex and exposure to TFA on alterations in these brain inflammatory target genes.

RESULTS: 102 animals (n= 46 TFA and 56 AIN-93 from 12 litters each) were analyzed (Table). Female sex was significantly associated with increased Ccl7 (chemokine specific for monocytes) and interleukin-1 (IL-1) receptor, type 2 (a decoy receptor for IL-1) expression, while decreased CCl7 expression was also significantly associated with TFA in utero exposure. Further, CxCl1 (chemokine specific for neutrophils) expression was associated with TFA in utero exposure in females, but not males. Other target genes evaluated (CCl11, Cx3Cl1, IL-10, IL-17B, and TGF-ß) did not show any significant relationships with sex or diet.

Target Gene Expression by Sex and Diet

Target Gene	TFA:AIN-93	Male:Female	Diet*Sex Interaction
Ccl7	0.50*	0.50*	No
Ccl11	0.80	0.69	No
CxCl1	2.00	1.00	Yes
Cx3Cl1	1.12	0.98	No
IL-1R2	1.00	0.63*	No
IL-10	1.00	2.00	No
IL-17B	1.00	0.50	No
TGF-ß	0.99	0.80	No

* p <.05 (expression in copies/µl normalized to ß-actin)

CONCLUSIONS: In utero exposure to trans fat exposure results in alterations in brain chemokine expression in offspring. As these molecules are known to play regulatory roles in inflammation and development of neural cell precursors, maternal diets enriched with trans fat may result in programmed alterations in neurological development.

T-101

Transcriptome Analysis of the Hypothalamus of 1-Year Old Male Mice Exposed during Early Development to Chronic Maternal Protein Deprivation. Alfred Balasa,[1] Christian L Dove,[2] Ignatia B Van den Veyver.[2,3] *[1]USDA/ARS Children's Nutrition Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; [2]Department of Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX, USA; [3]Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, USA.*

We have previously shown that offspring exposed to chronic maternal low protein diet (MLP) in development have significantly lower body weights up until 1 year of age (P365), lower standardized hind-leg muscles weights at 21 days (P21) and P365, and decreased serum levels of liver enzymes (ALT, LDH) at P365. Gene expression profiling of liver at P365 revealed a significant over-expression of the cohesin-mediator complex, but we found no differences in gene expression in the skeletal muscle of the P21 and P365 mice. To further understand the origins of the observed body-weight phenotype and examine a role for cohesins in other organs, we examined if chronic maternal protein deprivation affects gene expression in the hypothalamus of offspring, in particular of genes controlling satiety, as an explanation to for long-term altered body weight.

We fed adult C57BL/6J dams an 8% protein diet (MLP) or 20% protein control diet (C) from four weeks prior to mating throughout lactation. Male pups were weaned to standard lab rodent diet and single-housed at P21. Weight changes were followed up to P365 and mice were sacrificed at P21 and P365 for organ dissection and molecular analysis. RNA from total hypothalamus at P365 was subjected to RT-qPCR for expression analysis of *Cartpt, Npy, Npy5r, Lepr, Mc4r, Pomc*. Affymetrix Gene 1.0 ST arrays were used for gene expression profiling on 3 MLP and 3 C hypothalami; data was analyzed using Partek Genomics Suite.

In contrast to liver we did not find differences in expression between hypothalami of P365 MLP and C mice for all examined genes in the orexigenic-anorexigenic pathway. There were also no global differences in the transcriptome profiles.

The absence of differences in expression in the examined genes of the P365 hypothalamus indicates that the observed phenotypes are not associated with long term gene expression changes in this organ. Future experiments will examine gene expression differences at the end of exposure (at P21, when mice are weaned) and will focus on regional differences in selected hypothalamic nuclei.

T-102

Programmed Impaired Immune System Function Induced by Maternal Undernutrition in Pregnancy and Lactation. Ron Beloosesky, Mina Desai, Makiko Yamada, Michael G Ross. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Newborn infant humoral and cellular immunologic mechanisms are immature, particularly during the first month of life. Fetal/newborn undernutrition may cause further deficiencies to the neonatal host defense mechanisms. We sought to determine the impact of undernutrion during gestation and lactation, or gestation alone, on offspring basal and lipopolysaccharide (LPS)-stimulated immune indices and inflammatory response in the neonatal period.

Methods: From 10 days of gestation to term, pregnant rats received either ad libitum (AdLib) feed or were 50% food restricted (FR). At 1 day of age, litter size was standardized (4 males and 4 females per litter) and pups were either nursed by their own dams or were cross-fostered resulting in three groups (n=6 per group): Control, food restriction during gestation alone (FR/AdLib) and food restriction during gestation and lactation (FR/FR). At 3 weeks of age, the rats were injected with either LPS (100 mg/kg bw) or saline, and blood collected and analyzed for TNF-α levels (ELISA) and white blood cells indices (WBC count, percentage of lymphocyte, neutrophil and monocyte) at 3 hours.

Results: At 3 weeks of age, basal TNF-α levels (~20pg/ml), total WBC count (~1.5*1000/ul), and percentage of lymphocyte (~80%) and neutrophil (~17%) were comparable in all three groups. However, monocyte percentage was significantly decreased in FR/FR vs FR/AdLib and Control males (1.6±0.5 vs 2.4±0.4 and 3.1±0.3%, respectively). In response to LPS, FR/AdLib and Controls showed similar response with increased TNF-α levels, decreased WBC and monocytes, and unchanged neutrophils and lymphocytes. In contrast, FR/FR males had suppressed inflammatory response as evident by reduced TNF-α levels as compared to FR/AdLib and Control (207±28 vs 512±91 and 484±59 pg/ml, respectively). Furthermore FR/FR percentages of monocytes (5.4±1 vs 2.0±0.5 and 2.0±0.7%) and neurophils (51±5 vs 17±2 and 20±4%) were markedly increased whereas lymphocytes (43±5 vs 80±2 and 77±4%) were decreased as compared to FR/AdLib and Control.

Conclusion: These findings suggest that early undernutrition, particularly during prenatal and postnatal periods, affects offspring immune competence by decreasing basal monocyte percentage and reducing cytokine induction to inflammatory stimuli.

T-103

Vulnerable Periods for Inducing a Decrease in Postnatal Weight Gain by Perinatal Stress and Dexamethasone (DEX) Exposure in Mice. Christoph Bergmeier, Otto W Witte, Matthias Schwab. *Department of Neurology, University Hospital Jena, Jena, Germany.*

Prenatal glucocorticoids (GC) to enhance fetal lung maturation threaten premature labor restrict fetal growth in humans, sheep, and rodents at birth (J Mat Fetal Med 1999, 8:81-7) whereas prenatal chronic variable stress induce a higher body weight (Physiol Behav 2006, 88:605-14). To date, only limited data on the exact vulnerable periods of GC exposure and postnatal weight gain are available.

Objective: To examine gender specific vulnerable periods for inducing a decrease in postnatal weight gain via perinatal stress and dexamethasone (DEX) exposure in mice.

Methods: 230 mice (CL57/BL6) were treated with saline or 2x170 µg/kg body weight DEX 24 hrs apart corresponding to a weight-adapted dose of 2x12mg DEX administered to a 70kg pregnant woman at either E14/15, E16/17 or E14-17 (term E19). Litter size was reduced to six offspring at birth to ensure equal conditions. An additional group of offspring of untreated mothers underwent postnatal stress at P3-13 via a 3h separation from mothers (PS). First measurements for body weight were undertaken at P3 to avoid rejection of the offspring by the mothers.

Results: At P3, body weights were comparatively similar between the groups. Weight gain did not differ significantly during the first 25 days. At P39, males of all DEX treated groups had a lower body weight than controls with the most pronounced effect in the group treated for 4 days (Table 1). Only the females treated for 4 days demonstrated this effect. In contrast, separation from mothers induced higher body weights in females but not in males. At 1.5y of age, the effect had vanished.

Conclusions: Prenatal DEX exposure during the last trimester of gestation led to a delayed weight gain after lactation independent of the time of administration. Male offspring were more sensitive. In contrast, early postnatal stress induced increased postnatal weight gain in females but not males. These effects did not last until old age.

	Female [g]		Male [g]	
controls	16.9±1.1		21.9±1.9	
DEX E14/15	17.5±1		20.5±1.1	p<0.005
DEX E16/17	16.9±1.7		20±3.3	p<0.005
DEX E14-17	14.7±1.0	p<0.001	17.8±1.9	p<0.05
postnatal stress	18.5±1.1	p<0.001	21.6±1.3	

Table 1: Body weights at P39.

T-104

Vulnerable Periods for Programming the Hypothalamic Pituitary Adrenal Axis (HPAA) by Perinatal Stress and Dexamethasone (DEX) Exposure in Female Rats. Christoph Bergmeier, Otto W Witte, Matthias Schwab. *Department of Neurology, University Hospital Jena, Jena, Germany.*

Prenatal glucocorticoids (GC) program HPAA activity and about 10% of all pregnancies threatened with premature labor are treated with synthetic GC to enhance fetal lung maturation. The vulnerability differs gender specific in guinea pigs (Am J Physiol 280:E729-39, 2001). However, there are only limited data on vulnerable periods of GC exposure in female rats.

Objective: To systematically examine vulnerable periods for programming HPAA activity in female rats by DEX at doses used clinically to enhance fetal lung maturation.

Methods: Wistar rats were treated with saline or 2x170 µg/kg body weight DEX 24h apart corresponding to the weight adapted dose of 2x12mg DEX administered to a 70kg pregnant woman at E15/16, E17/18, E19/20 or P4/5 (term E21). Litter size was reduced to 10 offspring at birth to ensure equal conditions. An additional group of offspring of untreated mothers underwent postnatal stress by a 3h separation from mothers at P3-13. At least 12 female offspring underwent the forced swimming test after estrus synchronization at 8 months of age. Serum corticosterone levels were estimated at baseline, on completing the swim test, and 1 and 2h after the test.

Results: The DEX E17/18 group had higher baseline corticosterone levels (Fig. 1). Rats in the DEX E15/16, E17/18, and E19/20 groups showed an increased and prolonged stress response with the highest corticosterone levels in the DEX E17/18 group. In contrast, the DEX P4/5 group demonstrated a decreased stress response. However, a separation from mothers at P3-13 also induced an increased stress response.

Conclusions: DEX exposure at E17/18 had the most pronounced effects with regard to inducing a hyper-responsive HPAA. In contrast to early postnatal stress, DEX exposure at P4/5 induced a hypo-responsive HPAA. Vulnerable periods for fetal HPAA programming by GC differ from those for development of hypertension (Kidney Int 59:1663–9, 2001).

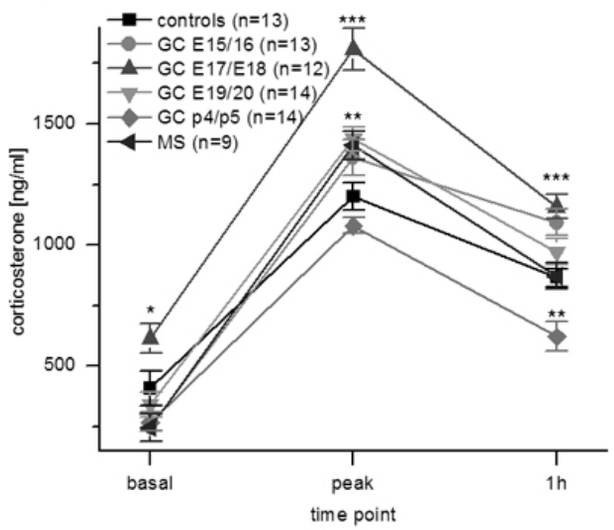


Fig 1: Corticosterone response to stress following perinatal GC exposure in female rats at 8mo of age. (*p<0.05,**p<0.01,***p<0.001) MS: mother separation

T-105

Regulation of Intramembranous Absorption and Amniotic Fluid Volume by Constituents in Fetal Urine and Amniotic Fluid. Robert A Brace,[1,2] J Job Faber,[2] Sonnet S Jonker,[2] Cecilia Y Cheung,[1,2] Debra F Anderson.[2] *[1]Obstetrics & Gynecology, Oregon Health & Science Univerity; [2]Heart Research Center, Oregon Health & Science University.*

Introduction

Amniotic fluid (AF) volume is determined by a balance between 2 primary amniotic inflows and 2 primary outflows. Recent studies have shown that intramembranous absorption (IMA) is regulated under several experimental conditions to maintain AF volume near normal. In contrast, fetal urine production, lung liquid secretion and fetal swallowing are regulated to maintain fetal body homeostasis rather than AF volume. It has been hypothesized, but not demonstrated, that chemical constituents in AF are the primary regulators of the rate of IMA and hence AF volume. In this study, we tested the hypothesis that fetal urine is the source of the AF chemical factors that regulate absorption of AF by the intramembranous pathway.

Methods

We measured AF volume at 2-3 day intervals and total volumes of fetal urine production, lung liquid secretion, and swallowed volume over the same time intervals in late gestation, chronically catheterized fetal sheep. The rate of IMA was calculated from the time integrated volume flows and the changes in AF volume.

Results

During control conditions when fetal urine entered the AF, IMA was 888 ± 95 (SE) ml/day and AF volume was 687 ± 201 ml. In response to fetal urine diversion to the exterior and isovolumic replacement with lactated Ringer's solution, IMA decreased to 303 ± 108 ml/day (P = 0.0034 compared to control) and AF volume increased to 1,540 ± 299 ml (P = 0.0068). In response to supplementing AF inflow with lactated Ringer's solution at 2 or 4 liters/day, IMA increased to 1,998 ± 299 ml/day (P = 0.0069 compared to control values) while AF volume increased to 2,914 ± 497 ml (P = 0.0008). Isovolumic fetal urine replacement during intra-amniotic supplementation resulted in no further change in IMA rate (2,168 ± 331 ml/day, P = 0.46) or AF volume (2,534 ± 560 ml, P = 0.47) compared to supplementation alone.

Conclusions

1) The decreased IMA during urine replacement suggests that constituents in fetal urine act as stimulators of IMA rate; 2) the increased absorption during intra-amniotic fluid supplementation likely is due to a non-renal inhibitor of absorption that is normally present in AF and is diluted by the infused fluid. We speculate that renal and non-renal stimulators and inhibitors of IMA are present in AF and they function competitively as primary regulators of AF volume.

T-106

Changes in Fetal Swallowing as a Protective Mechanism Against Abnormal Amniotic Fluid Volumes. Robert A Brace,[1,2] Cecilia Y Cheung,[1,2] Debra F Anderson.[2] *[1]Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR; [2]Heart Research Center, Oregon Health and Science University, Portland, OR.*

Objective

Late gestation fetuses normally swallow large volumes of amniotic fluid (AF) daily. However, the extent to which swallowed volume depends on AF volume is unknown and it is unknown whether the AF volume induced changes in swallowed volume may compensate for aberrant AF volumes. Our objective was to characterize fetal swallowing of AF over a wide range of AF volumes.

Methods

AF volume was measured at 1-3 day intervals in late gestation ovine fetuses during control conditions, fetal hypoxia, and intra-amniotic fluid infusion. Swallowed volume was determined by integrating the flow signal from a flow probe around the fetal esophagus.

Results

For all data combined, AF volume ranged from 160 ml to 6150 ml and swallowed volume from to 36 ml/day to 1,963 ml/day. Under control conditions, AF volume averaged 652 ± 113 (SE) ml, swallowed volume 411 ± 40 ml/day, and 75.1% ± 9.3% of the swallowed volume was ingested during 5.7 ± 1.8 bouts/day of rapid swallowing. Swallowed volume was near zero when AF volume was far below normal, at its maximum of 635 ± 41 ml/day when AF volume was 1,682 ± 31 ml, and was unchanged with higher AF volumes. The relationships between swallowing and AF volume were similar during normoxia, long-term hypoxia, and long-term intra-amniotic fluid infusion. The number of bouts of swallowing/day correlated positively with AF volume (r = 0.95, P<0.0001) and increased to a maximum of 14 ± 2 bouts /day when AF volume exceeded 2,000 ml. However, the volume swallowed per bout

(57.3 ± 5.8 ml) was independent of AF volume. The number of swallows/day, the mean volume/swallow and the mean duration of a swallow correlated positively with AF volume.

Conclusions

The volume of AF swallowed each day by the fetus 1) is a strong function of AF volume; 2) is similar under several long-term stable conditions; and 3) is a maximum when mild polyhydramnios develops and does not increase with further increases in AF volume. 4) Changes in the volume swallowed/day are mediated primarily by alteration in the number of bouts of swallowing/day. 5) The passive effects of uterine compression of the fetus may mediate the alterations in swallowed volume that occur when AF volume changes; and 6) The AF volume induced changes in daily swallowed volume significantly reduce the extent of oligohydramnios and polyhydramnios that would otherwise occur.

T-107

Antioxidant Capacity of Adipose Tissue in Offspring Exposed to Prenatal High Fat Diet. Egle Bytautiene, Talar Kechichian, Esther Tamayo, Phyllis Gamble, Monica Longo, George R Saade. *Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective:

Obesity is characterized by adipose tissue accumulation, which in turn is associated with metabolic consequences. The objective of this study was to investigate the antioxidant capacity of visceral adipose tissue in an animal model of developmental programming of metabolic syndrome.

Study design:

CD-1 female mice were placed on standard (SF) or high fat (HF) diet starting 3 months before mating. The offspring from both groups of dams were placed on SF diet after weaning. At 6 months of age, the offspring were euthanized and tissues collected. Protein expression of Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) in visceral adipose tissue and kidney were determined using Western blot analysis (SF males n=4-5, females n=5; HF males n=5, female n=5). Results were analyzed using Students t-test or Mann Whitney test (significance $p<0.05$).

Results:

SOD (p=0.02), GPx (p=0.04), CAT (p=0.03) expressions were significantly lower in adipose tissue from males born to high fat diet fed mothers when compare to SF group. There was a tendency for lower expression of SOD, GPx and CAT in female offspring from high fat diet mothers. No statistically significant differences in SOD, PGx, and CAT expressions were determined in kidney from HF males and females compared to SF group.

Conclusions:

Maternal pre-pregnancy obesity from high fat diet programs the offspring for lower depots of antioxidant enzymes in adipose tissue. The altered antioxidant status may represent an additional factor towards the development of metabolic syndrome and related complications.

T-108

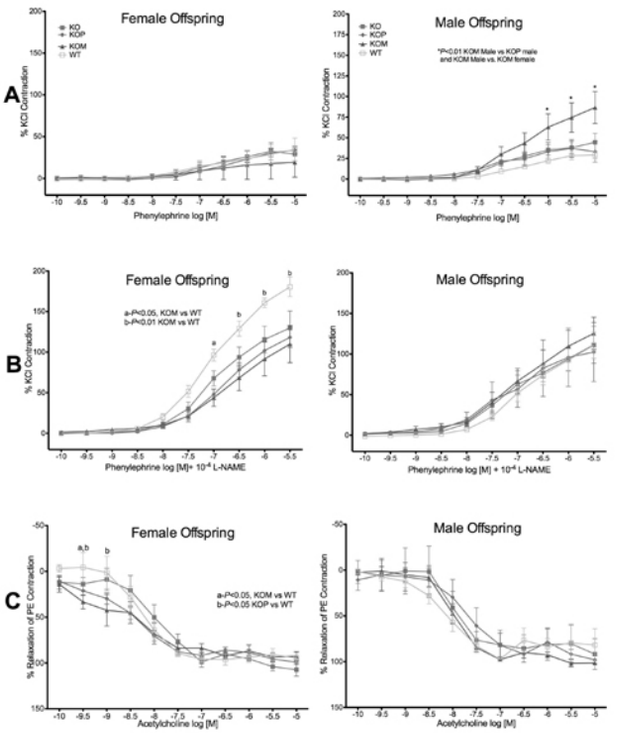
Effect of Early Postnatal Environment on Developmental Programming of Vascular Reactivity in a Mouse Model of Hyperlipidemia. Karin Fox,[1] Danielle Schwartzenburg,[2] Egle Bytautiene,[1] Esther Tamayo,[1] George R Saade,[1] Nima Goharkhay.[1] *[1]Department of OB-GYN, University of Texas Medical Branch, Galveston, TX, USA; [2]NIDDK Research Enrichment and Preparation Training Program, NIH, School of Medicine, University of Texas Medical Branch.*

Objective: We have shown that heterozygous apoE+/- offspring born to apoE-/- dams (maternally-derived heterozygous; KOM) develop more severe hypercholesterolemia and atherosclerosis than their genomically-similar apoE+/- offspring born to wild-type apoE+/+ dams (paternally-derived heterozygous; KOP), which was mitigated by cross-fostering. In naturally-fostered offspring, blood pressure and vascular reactivity were similar in all groups. The aim of this study was to determine long-term effects of cross-fostering on vascular reactivity.

Study Design: Homozygous apoE -/- knockout (KO) and wild-type (WT) mice were cross-bred to obtain genomically similar heterozygous offspring with a paternally-derived (KOP) or maternally-derived (KOM) knockout allele, and homozygous WT & KO litters. After delivery KOM & KO litters were placed with a WT dam; KOP & WT litters were placed with a KO dam. All pups ate regular chow after weaning. At 8 months, carotid arteries were collected, mounted on a wire myograph (*n*=6-15/group), and contractile response curves to phenylephrine (PE) or PE plus L-NAME and relaxation responses to acetylcholine (Ach) were recorded. Two-way ANOVA with Bonferroni multiple comparisons were used for statistical analysis (significance: $P<0.01$).

Results: Arteries from KOM males contracted significantly more with PE than those from KOM females and KOP males (figure, panel A). In the presence of L-NAME, WT females exhibited increased contractility (figure, panel B). Relaxation responses were similar among all groups.

Conclusions: Although normal environment during the early postnatal period mitigates the adverse effect of intrauterine programming on serum lipid levels and atherosclerosis in offspring, this effect does not appear to be mediated by changes to vascular reactivity. Cross-fostering shows moderate gender specific effects on vacsular response to phenylephrine in this model.



T-109

Prenatal Risks for Neurological Disease: Role of Hypoxia and Oxidative Stress. EJ Camm,[1] D Tijsseling,[2] CM Lusby,[1] AD Kane,[1] JB Derks,[2] DA Giussani.[1] *[1]Physiology, Development & Neuroscience, University of Cambridge, United Kingdom; [2]Obstetrics, University Medical Center, Utrecht, Netherlands.*

Clinical and experimental data have shown that many major diseases of later life, including hypertension and type 2 diabetes, are developmental in origin and can arise from nutritional imbalance during pregnancy. It is unclear whether fetal hypoxia, as occurs in preeclampsia or placental insufficiency, can also programme neurological impairment. This interventional study tested the hypothesis that developmental programming of neurological disease by prenatal hypoxia does occur, and that this is secondary to oxidative stress. We investigated in rats the effects of prenatal hypoxia on behaviour and cognitive function in adult offspring, and determined whether allopurinol had any neuroprotective effects.

METHODS: Pregnant Wistar rats were exposed to normoxic (21% O2) or hypoxic conditions (13% O2) +/- allopurinol (30mg/kg/day in jelly) from days 6-21 of gestation. At birth, litters were culled to 8 pups, and offspring group housed until adulthood. At 3.5 months, anxiety and cognitive functions were assessed using an elevated plus maze and object recognition (OR) task, respectively (n=9-11, 1 male per litter per group).

RESULTS: Relative to controls (body weight, 566±12; brain weight, 2.05±0.03g), hypoxic pregnancies with or without allopurinol did not alter the body or brain weights in adult offspring. The measure of OR, the discrimination ratio (DR), was significantly reduced 3 hours after testing in adult offspring of hypoxic pregnancy compared to controls (Fig. 1). This ratio remained attenuated 24 hours after testing in offspring of hypoxic pregnancy, suggesting long-term memory impairment. These adverse effects were absent in offspring of hypoxic pregnancy treated with allopurinol. Performance in the elevated plus maze was not different between the groups.

CONCLUSIONS: Memory impairment in adulthood programmed by prenatal hypoxia can be ameliorated by allopurinol treatment. Xanthine oxidase-mediated oxidative stress may be a key link in the developmental origins of neurological disease.

Supported by the BHF and BBSRC

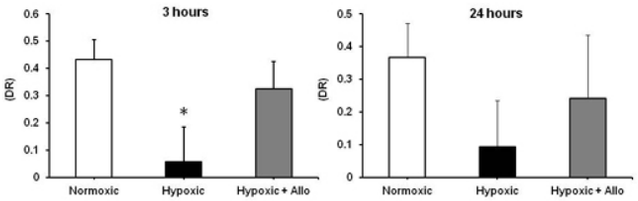


Fig. 1. The discrimination ratio (DR) at 3 and 24 hours for the object recognition (OR) task. Values are mean ± SEM in normoxic (□), hypoxic (■) and hypoxic + allo (▩) offspring. Significant differences * P<0.05, one-way ANOVA with *post hoc* Tukey.

T-110

Gender Difference in Renal ACE and ACE2 Activity Development in Sheep. Kai Chen, Jianli Bi, James C Rose. *OB/GYN, Wake Forest University School of Medicine.*

Objective: Renin angiotensin system (RAS) is important in hemodynamic regulation, and kidney development. ACE and ACE2 are two key enzymes of intrarenal RAS. Previously we showed that antenatal glucocorticoid treatment of pregnant sheep induced ACE and ACE2 activity changes in their offspring. This study was designed to investigate the normal developmental change of renal ACE and ACE2 activity in both male and female sheep.

Methods: 4 groups of date-mated sheep were studied (fetuses (135 days of gestation age), new born (NB) at 5-7 days, adults at 1 year old (1yo) and two year old (2yo)). Sheep were anesthetized and nephrectomy was performed, the kidney cortex was dissected on ice and frozen at -80 ºC for further study. ACE and ACE2 activities (expressed as fmol/min/mg protein) were assayed by radioactivity assay. All data were expressed as the means±SEMs. Two way ANOVA was used for developmental data analysis and t test for gender data in the different age groups. P<0.05 was used as statistical significance.

Result:

Table 1:

		135 GA	New Born	1 year old	2 year old
Male	ACE	654.0±336.6	594.8±46.3	1247.0±77.1*+	303.3±118.4+
	ACE2	333.0±75.7#	429.4±8.3	1580.0±129.4*#	721.2±86.4#
	ACE/ACE2	2.14±1.09	1.40±0.13	0.82±0.08*	0.44±0.18*
Female	ACE	28.2±12.2	690.2±48.7	568.2±196.3	1275.0±101.0*
	ACE2	1171.0±56.5	482.6±62.88	690.8±163.2	1826.0±312.6*
	ACE/ACE2	0.02±0.01	1.47±0.12*	1.00±0.33	0.84±0.13

1. Males reached highest ACE and ACE2 activity at 1 yo and then deceased (*p<0.05 vs 135GA, NB, 2yo). Females reached highest ACE and ACE2 activity level at 2 yo (*p<0.05 vs 135GA, NB,1yo). 2. In males, the ACE/ACE2 ratio was decreased in adult (*p<0.05 vs. 135GA). In females, the ratio was higher in NB compared to 135GA, with no significant change after birth (*p<0.05 vs. 135GA). After birth, the ratio trended down in both males and females and there was no gender difference. 3.Gender difference include males with higher ACE activity at 1 yo but lower activity at 2 yo (+p<0.05 vs. female) and lower ACE2 activity at 135GA, higher at 1 yo and then lower again at 2 yo (#p<0.05 vs. female).

Conclusion:

1. Developmental changes in renal ACE activity, ACE2 activity and ACE/ACE2 activity ratio have gender differences in sheep. 2. These gender differences may indicate different renal development, maturation or function. 3. The physiological consequences of gender specific developmental changes in renal ACE and ACE2 activity needs further investigation.

T-111

New Insights into Amniotic Fluid Prostaglandin E_2: Sources and Relationship with Amniotic Fluid Volume. Cecilia Y Cheung,[1] Michael K Beardall,[1] Debra F Anderson,[2] Robert A Brace.[1] *[1]Obstetrics and Gynecology, Oregon Health and Science University; [2]Heart Research Center, Oregon Health and Science University, Portland, OR, USA.*

Introduction: Amniotic fluid (AF) prostaglandin (PG) E_2 may mediate a variety of biological functions including inflammation, initiation of labor and AF volume regulation. However, contributors to the AF PGE_2 pool or determinants of its concentration have not been established. Potential sources include the fetal kidneys, lungs and membranes. The objectives of this study were to determine PGE_2 concentrations in the AF and the contribution of urine and lung liquid PGE_2 to AF levels under conditions of normal and altered AF volumes. We hypothesized that AF contains high levels of PGE_2 derived primarily from fetal urine and that AF concentration is correlated with AF volume.

Methods: Eight near-term fetal sheep were studied. AF volume was modified either by fetal urine diversion plus isovolumic replacement with lactated Ringer's solution or intra-amniotic supplementation with Ringer's at 2L/day. In some fetuses indomethacin (10 mg/day) was continuously infused intravenously. At 48 hours, steady state samples of AF, fetal urine and lung liquid were collected for PGE_2 analysis by ELISA. AF volume was measured by drainage and intramembranous absorption rate (IMA) of AF calculated from time integrated flows and volumes.

Results: Control PGE_2 concentration in the AF was 734±123 (SE) pg/ml while urine and lung liquid levels were 1,501±245 pg/ml and 101±25 pg/ml, resp. Urine diversion plus replacement reduced AF PGE_2 to 334±78 pg/ml. Unexpectedly, fluid supplementation, instead of diluted, elevated AF PGE_2 to 1,190±603 pg/ml (P<0.0001, compared to control and diversion). Control AF volume was 687±201 ml and IMA 888±95 ml/day. Urine diversion plus replacement increased AF volume to 1,540±299 ml due to a fall in IMA (303±108 ml/day). Supplementation further increased AF volume to 2,914±479 ml (P<0.01) and IMA to 1,988±289 ml/day (P<0.0001). Indomethacin significantly reduced AF, urine and lung liquid PGE_2 concentrations to 30±7% of control. AF PGE_2 concentration was not correlated with AF volume.

Conclusion: When flow and concentration were taken into account, fetal urine PGE_2 contributes approximately 40% to the AF PGE_2 pool while lung liquid contributes to a much lesser extent (<5%). We speculate that the fetal membranes may be an important source of AF PGE_2 under normal conditions and especially when AF volume is expanded.

T-112

Pregestational and Prenatal Maternal Stress Exposure Do Not Predict Increased Risk for Infant Mortality. Quetzal A Class,[1] Khashan S Ali,[2] Brian M D'Onofrio,[1] Langstrom Niklas,[3] Lichtenstein Paul.[3] *[1]Psychological and Brain Sciences, Indiana University; [2]Anu Research Centre, University College Cork; [3]Medical Epidemiology and Biostatistics, Karolinska Institute.*

Infant mortality (IM) has great psychological and financial burden at both the individual and societal level. Adverse birth outcomes are consistently associated with increased risk for IM. Due to the associations found between pregestational and prenatal maternal stress and adverse birth outcomes, it may be that stress exposure either directly, or through a pathway mediated by adverse birth outcomes, influences the risk for IM.

Using a population-based register of all children born in Sweden from 1973-2008 (N=2,988,093), we identified 8,447 cases of IM. Stress was indicated by death of a first degree relative of the mother during 6 mo prior to conception (pregestation; 20,400) or prenatal development (26,526). First degree relatives included the parent, sibling, spouse, or already born child of the mother. Logistic regression accounting for family clustering was used to predict adjusted odds ratios (OR) for IM. Adjusted models accounted for target child sex, year of birth, and birth order as well as parental age, highest education, and Swedish or non-Swedish birth. Mediation by preterm birth and low birth weight was investigated.

No increased risk for IM was found following prenatal maternal stress exposure (OR = 1.04, 95% CI 0.84-1.30). Increased risk following pregestational stress was identified (OR = 1.53, 95% CI 1.26-1.87) and did not appear to be mediated by adverse birth outcomes (OR = 1.34, 95% CI 1.09-1.65). After adjusting for interpregnancy interval and age at which the already born child died, the association between pregestational stress and IM was no longer present (OR = 0.96, 95% CI 0.77-1.20). In this model, shorter interpregnancy interval (OR = 1.27, 95% CI 1.19-1.35) and younger age of the already born child that may have died (OR = 1.70, 95% CI 1.56-1.85) were significant predictors of the target child's IM.

Findings suggest that prenatal maternal stress is not associated with increased risk for IM. Therefore, adverse birth outcomes may be associated with IM for reasons other than prenatal maternal stress exposure. Additionally, the results provide evidence in support of a familial risk for IM and may suggest that selective fertility factors influence the length of interpregnancy interval.

T-113

Novel Genetic Associations with Compromised Metabolic Phenotypes in a Mouse Model of Maternal Undernutrition. KL Connor,[1] E Matysiak,[1] L Chun,[1] B Knight,[1] CE Pennell,[2] SJ Lye.[1] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hospital, Toronto, Canada; [2]School of Women's & Infants' Health, Univ West. Australia, Perth, Australia.*

Mounting evidence demonstrates that both the early life environment and genetic factors influence later health and disease risk. Previously we showed that maternal dietary restriction (DR) from mid gestation results in obesity and impaired glucose tolerance in adult offspring, dependent upon offspring sex and genetic background. We hypothesised that these metabolic phenotypes may be associated with changes in hepatic triglyceride (TG) deposition and key genes associated with obesity, insulin resistance and fatty liver disease.

A/J and C57BL/6J (B6) female mice were mated with males of the same strain. Pregnant females were randomised to either control chow ad libitum throughout pregnancy (CON), or dietary restriction by 30% from days 6.5 to 17.5 of pregnancy (DR), after which dams and offspring were fed chow ad libitum, creating four groups: B6 CON; B6 DR; AJ CON; AJ DR. At 6 months postnatal age, offspring were killed and livers collected for histopathology and to determine TG content and mRNA expression of: fat mass and obesity related gene (FTO), TG metabolism patatin-like phospholipase domain-containing protein 3 (PNPLA3), vitamin D binding protein (GC) and lymphocyte cytosolic protein 1 (LCP1). Hepatic TG content was analysed by ANOVA and qPCR data were analysed by Pfaffl's relative ratio; $p<0.05$.

In B6 males, DR resulted in lower hepatic FTO, GC and LCP1 mRNA expression compared to B6 CON males, whilst AJ DR males had increased GC and PNPLA3 mRNA expression, and AJ CON males had increased FTO, GC and PNPLA3 mRNA expression, compared to B6 CON. In B6 females, DR resulted in lower hepatic FTO, GC and LCP1 mRNA expression compared to B6 CON females, whilst both AJ DR and AJ CON females had lower FTO and LCP1 mRNA expression compared to B6 CON. In both males and females, there was no difference between groups in hepatic TG content or histopathological markers of fatty liver disease.

Our results show that in adult mice, altered hepatic expression of key genes associated with obesity, insulin resistance and fatty liver disease is dependent upon maternal nutritional history, offspring sex and genetic background, and is consistent with a phenotype of metabolic dysfunction in these offspring. Importantly, these genes may serve as early markers of a compromised liver phenotype prior to the onset of fatty liver disease.

T-114

Maternal Glucocorticoid Treatment Modifies the Fetal Methylome and Alters Expression of Key Methylation-Related Enzymes in a Tissue-Specific Manner. A Crudo,[1,2] A Kostaki,[3] V Moisiadis,[3] M Szyf,[1,2] SG Matthews.[3,4,5] *[1]Pharmacology & Therapeutics, Mcgill University, Canada; [2]Sackler Program for Epigenetics & Psychobiology, McGill University; [3]Physiology, ; [4]Obstetrics & Gynecology, ; [5]Medicine, University of Toronto, Canada.*

Objective: Fetal endogenous glucocorticoids (GC) increase exponentially in late gestation, and act to mature several organ systems. Thus, synthetic glucocorticoids (sGC) are given to pregnant women at risk of pre-term delivery. In animal studies, prenatal sGC exposure can lead to modification of endocrine function and behavior in offspring; this involves long-term changes in gene expression. We hypothesized that:1) the natural GC surge is associated with DNA methylation changes in the fetal epigenome, and sGC treatment prematurely initiates this process;2) early exposure to sGC alters the expression of genes known to regulate methylation.

Methods: Pregnant guinea pigs (F0; n=4) were s.c. injected with betamethasone (BETA; 1mg/kg), a sGC, on gestational days (GD) 40, 41, 50 & 51, or received no treatment. Control and treated animals were euthanized on GD52 (prior to the cortisol surge) and on GD65 (post surge). Global methylation was examined with the Luminometric Methylation Assay (LUMA). mRNA levels for genes involved with epigenetic regulation were examined using qRT-PCR.

Results: With advancing gestation, there was a significant decrease in global methylation of adrenal and placenta, and an increase in global methylation in the kidney ($P<0.05$). At GD52, BETA significantly decreased global methylation in the liver, adrenal and placenta, and increased it in the kidney ($P<0.05$). At GD65, global methylation was significantly decreased in sGC-exposed placenta, but was increased in the liver and adrenal ($P<0.05$). mRNA expression analysis in the kidney and placenta showed a significant effect of treatment and age on mRNA levels of genes involved in epigenetic regulation, including, Dnmt1, Dnmt3b and Mbd2.

Conclusion: There are substantial global methylation changes in fetal tissues in late gestation, which coincide with the endogenous cortisol surge. While antenatal sGC treatment may accelerate the developmental state of methylation, its effects are maintained and further modify the epigenome with advancing gestation. Exposure to synthetic or natural GC modifies the epigenetic machinery by modifying the expression of genes that regulate the epigenome.

Supported by: The Canadian Institutes of Health Research

T-115

Glucocorticoids Program the Fetal Hippocampal Epigenome. A Crudo,[1,2] M Suderman,[1,2] A Kostaki,[3] V Moisiadis,[3] M Szyf,[1,2] SG Matthews.[3,4,5] *[1]Pharmacology & Therapeutics, McGill University, Canada; [2]Sackler Program for Epigenetics & Psychobiology, McGill University; [3]Physiology, ; [4]Obstetrics & Gynecology, ; [5]Medicine, University of Toronto, Canada.*

Objective: Fetal endogenous glucocorticoids (GC) increase exponentially in late gestation, and act to mature several organ systems. Thus, synthetic glucocorticoids (sGC) are given to pregnant women at risk of pre-term delivery. Animal studies revealed that prenatal sGC exposure can lead to modifications of endocrine function and behavior in offspring, which involve long-term changes in gene expression. We hypothesized that the endogenous GC surge is associated with the epigenetic programming of genes and that sGC treatment prematurely initiates this process.

Methods: Pregnant guinea pigs (F0; n=4) were s.c. injected with betamethasone (Beta; 1mg/kg), on gestational days (GD) 40, 41, 50 &51, or received no treatment. Groups of control and treated animals were euthanized on GD52 (prior to the cortisol surge) and on GD65 (post cortisol surge). Hippocampus was collected from male fetuses. Genome wide methylation and acetylation was examined using methylated DNA immunoprecipitation (MeDIP) and H3K9 acetylation chromatin immunoprecipitation (chIP) coupled with high-density oligonucleotide arrays. Probe intensities were extracted from hybridization images and analyzed.

Results: The natural cortisol surge was associated with increased methylation of 2000 genes. sGC exposure in GD52 animals resulted in hypermethylation in several thousand genes, and increased acetylation in fewer genes. However, comparison of hippocampi from sGC exposed GD52 animals to animals after the natural cortisol surge (GD65), showed similar patterns of methylation, suggesting sGCs prematurely modifies the epigenome. Further, BETA exposed GD65 hippocampi had hypermethylation in over 1500 genes, and a significant reduction in acetylation in ~500 genes.

Conclusion: This study demonstrates that the endogenous cortisol surge is associated with gene specific changes in the hippocampal epigenome. Further, premature exposure to sGC accelerates the methylation and acetylation profiles of gene promoters observed in normal development. These studies indicate dramatic changes in the fetal hippocampal epigenome in late gestation and that treatment with sGC has profound effects on the fetal epigenome. These changes likely have a major effect on gene transcription in the hippocampus.

Supported by:The Canadian Institutes of Health Research

T-116

Inhibition of the Nutrient Sensor SIRT1 Normalizes Neural Stem Cell Proliferation and Differentiation in Low Birth Weight Newborns. Mina Desai, Tie Li, Michael G Ross. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

OBJECTIVE: Maternal perinatal undernutrition results in low birth weight (LBW) newborns that exhibit hyperphagia and develop adult obesity. LBW-mediated hyperphagia results from reduced hypothalamic anorexigenic cellular signaling responses, suggesting altered neurodevelopment. Further, the hypothalamic neural stem cells (NSCs) from LBW newborns exhibit programmed reduction in proliferation and differentiation, potentially regulated epigenetically by upregulation of the hypothalamic nutrient sensor, SIRT1, a histone deacetylase. We sought to confirm the role of SIRT1 in NPC proliferation and differentiation using SIRT1 modulators.

METHODS: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term, resulting in LBW newborns. Hypothalamic NSCs were obtained from 1 day old LBW and Control newborns and cultured in complete medium. On day 3, cells were treated with SIRT1 activator (20μm resveratrol) or inhibitor (20μm sirtinol) for 5 days. SIRT1 expression, cell proliferation (MTT assay), and protein expression of markers of NSC (nestin), proliferation (Hes1), neurons (Tuj1) and astrocytes (GFAP), including were determined. Values were compared to untreated Control NSC.

RESULTS: SIRT1 activator (resveratrol) increased whereas inhibitor (sirtinol) decreased SIRT1 expression in both LBW (by 45% and 13%, respectively) and Control (by 30% and 25%, respectively) NSCs. Resveratrol inhibited NSC

proliferation with decreased nestin, Hes1 and Tuj1, and increased GFAP protein expression. In contrast, sirtinol promoted NPC proliferation with increased nestin, Hes1 and Tuj1, and decreased GFAP protein expression. At all times, LBW NSC maintained upregulated SIRT1 with decreased NSC proliferation, nestin, Hes1 and Tuj1, and increased GFAP expression, as compared to controls. CONCLUSION: In LBW newborns, reduced neurogenesis is epigenetically mediated via histone (SIRT1) modifications. We speculate that increased SIRT1, which reduces Hes1, induces premature differentiation to astrocytes and reduces the hypothalamic NSC pool in LBW offspring.

T-117

Epigenetic Mediated Early Induction of Adipocyte Differentiation Contributes to Programmed Obesity in Intrauterine Growth Restricted Newborns. Mina Desai,[1] Robert H Lane,[2] Guang Han,[1] Thomas R Magee,[1] Michael G Ross.[1] [1]*Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA;* [2]*Pediatrics, University of Utah, Salt Lake City, UT, USA.*

OBJECTIVE: A key feature of gestationally programmed obesity in intrauterine growth restricted (IUGR) newborns is enhanced adipogenesis. Adipogenesis is driven by adipocyte differentiation, a process whereby previously silent adipogenic genes are activated, in part, via epigenetic mechanisms. DNA methyltransferase (DNMT3a) and histone deacetylase (HDAC1) both suppress gene expression. We have previously shown that maternal food restriction results in IUGR newborns that develop adult obesity. Notably at 1 day of age, IUGR newborns have upregulated expression of adipogenic transcription factors (PPARγ, C/EBPα . We hypothesized that IUGR adipocytes exhibit enhanced adipocyte differentiation as a result of epigenetic mediated enhanced induction of adipogenic genes. Using primary adipocyte cultures, we determined the degree of induction of epigenetic modulators, adipogenic transcription factors and their downstream lipogenic target genes (SREBP1, fatty acid synthase, acetyl-CoA carboxylase) in IUGR and Control offspring.

METHODS: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term, resulting in IUGR newborns. Adipose tissue was obtained from 1 day old IUGR and Control newborns and cultured for 48h (time 0), at which time cells were induced to differentiate. Protein was extracted at day 0, 2, 4 and 6 and expression of epigenetic (DNMT3a, HDAC1), adipogenic (PPARγ, C/EBPα , and lipogenic factors (SREBP1, fatty acid synthase, acetyl-CoA carboxylase) were determined. Values were normalized to GAPDH and presented as fold change. RESULTS: In IUGR and Control adipocytes, prior to induction at day 0, DNMT3a and HDAC1 were highly expressed, in association with absent expression of adipogenic and lipogenic factors. With induction, IUGR DNMT3a and HDAC1 decreased by 90%, while Control DNMT3a and HDAC1 decreased minimally (30-40%). IUGR demonstrated greater expression of adipogenic (PPAR 2.5 vs 1.8 fold and lipogenic genes (SREBP1 2.2 vs 1.8 fold), which also occurred earlier in IUGR (peak value at 4 day) as compared to Controls (peak value at 6 day).
CONCLUSION: Enhanced induction of adipogenic genes as a result of highly suppressible DNMT3a and HDAC1 likely contributes to increased adipogenesis and obesity in IUGR offspring.

T-118

Altered Renal Gene Expression in Both IUGR and Non-IUGR Offspring from Nutrient-Restricted Ewes. Kathrin A Dunlap,[1] Rebecca M Simmons,[1] Michael G Uzelac,[1] Sorin M Greff,[1] Michael C Golding,[2] Michael C Satterfield.[1] [1]*Animal Science, Texas A&M University, College Station, TX, USA;* [2]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX, USA.*

Insufficient delivery of nutrients to the developing fetus results in intrauterine growth restriction (IUGR). In addition to increased risk of perinatal morbidity and mortality, IUGR is associated with increased risk of cardiovascular and metabolic diseases later in life. To determine if non-IUGR fetuses from nutrient-restricted (NR) ewes were molecularly similar to normal fetuses from controls we conducted the following study. Singleton pregnant ewes (n=24) of similar weight and body condition were fed 50% of their nutrient requirements from Days 35-125 of gestation. Ewes fed 100% of their nutritional requirements served as controls (n=7). Ewes were necropsied on Day 125 and fetuses weighed and dissected. Regression analysis found no correlation between maternal and fetal weight therefore, tissues from the six heaviest (NR non-IUGR) and six lightest (IUGR) fetuses from NR ewes, and tissues from all control fetuses were utilized for further study. Weights of IUGR fetuses were less (P<0.05) than those for NR non-IUGR and control fetuses (2.8±0.1 vs 4.1±0.1 vs 4.0±0.1, respectively). Absolute weights of the liver and kidney were lower (P<0.05) in IUGR fetuses versus NR non-IUGR and control fetuses, however when adjusted for fetal weight only the liver was disproportionately smaller in IUGR fetuses. Differences in gene expression of two physiologically important gene families were investigated to predict future growth and health. Analysis of the insulin-like growth factor (IGF) family found that circulating IGF1 levels were lower (P<0.05) in IUGR than NR non-IUGR and control fetuses. Conversely, hepatic IGF1 receptor mRNA expression was higher (P<0.05) in IUGR versus NR non-IUGR and control fetuses. We next analyzed components of the renin-angiotensin signaling family and discovered that renal angiotensin converting enzyme (ACE) mRNA levels were lower (P<0.05) in both IUGR and NR non-IUGR fetuses than controls. These results suggest that despite exhibiting a level of growth similar to controls, NR non-IUGR fetuses have a unique gene expression pattern within critical molecular pathways that may contribute to the development of cardiovascular disease while sparing the offspring from other metabolic consequences associated with being both NR and IUGR.

T-119

Sex Differences in Cytosine Methylation Associated with Abnormal Fetal Growth. Fabien Delahaye,[1] Hye J Heo,[1] Cristina Montagna,[2] Deyou Zheng,[2] John M Greally,[2] Francine H Einstein.[1] [1]*Department of Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, USA;* [2]*Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA.*

Objective: The epigenome may act as a biological memory of sub-optimal in utero conditions. We sought to determine if abnormal fetal growth is associated with modifications in genome-wide cytosine methylation profiles in a single population of multipotent hematopoietic stem cells.
Methods: Cord blood was collected from 3 groups of neonates (n=19/gp): 1) IUGR-birthweight (BW) and ponderal index (PI) <10th percentile 2) LGA- BW and PI>90th percentile 3) Con-BW and PI>10th and <90th percentile. CD34+ cells were isolated by immunomagnetic selection. For quantitative, genome-wide assessment of cytosine methylation, we used the Massively Parallel Sequencing-based HELP assay, which detects cytosine methylation levels at >1.8 million unique loci in the human genome. HELP assay compares HpaII and MspI genomic representations to identify hypomethylated and methylated loci. Loci of Interest (LOI) were based on strict criteria of two-sided t-test for locus-specific difference in average methylation between groups (p<0.0001) and magnitude of methylation difference between group means (angle >30 or <-30).
Results: As expected, BW and PI were statistically different among groups, but gestational age was similar. LGA mothers were older, had higher BMI at term and greater parity compared to other groups. The numbers of LOI identified for each comparison is shown in Table 1. Notably, greater numbers of LOI were identified when Con v IUGR males (533) and Con v LGA females (236) are compared, while much fewer were noted when Con v LGA males (80) and Con v IUGR females (7) were compared.

Number of Loci of Interest in Comparison Groups

Group 1	Group 2	Hypermethylated in Group2	Hypomethylated in Group2	Total
Con Male	IUGR Male	526	7	533
Con Female	IUGR Female	2	5	7
Con Male	LGA Male	53	27	80
Con Female	LGA Female	223	13	236
IUGR Male	LGA Male	0	194	194
IUGR Female	LGA Female	228	9	237

Conclusion: These findings suggest sex-specific differences in cytosine methylation dysregulation associated with abnormal fetal growth with a greater impact seen in IUGR males and LGA females. Further studies are needed to determine if the sets of loci identified are functionally significant or predictive of an adult phenotype, which is more susceptible to age-related diseases.

T-120

Cortisol Increases Activated Caspase-3 in Conductive Cells in the Fetal Heart. Xiaodi Feng, Maureen Keller-Wood. *Dept of Pharmacodynamics, University of Florida, Gainesville, FL, USA.*

We have previously found that increases in maternal cortisol in late gestation lead to a thickening of the wall of the fetal heart. Treatment of pregnant ewes with iv infusion of cortisol from 120 to 130 days gestation resulted in a statistically significant enlargement of the heart as measured by heart weight to body weight ratio (15%) or septal, right and left ventricular wall thicknesses (20%, 20% and 21%, respectively). This was associated with an increase in Ki67 staining in the heart (RV: 1.52±0.18 vs 0.95±0.09; LV: 1.66±0.19 vs 0.86±0.17 % of nuclei), and an increase in apoptosis, as measured by activated caspase-3 staining. In the case of caspase-3 staining, there was marked localization within

the subendocardial layers of the left and right ventricle, including the septum (eg in LV, control: 0.027±=0.008, cortisol: 0.113±0.028; in septum, control: 0.030±0.013 vs cortisol: 0.118±0.030 % area stained). In order to identify cell types positive for activated caspase-3, we have used co-staining of activated caspase-3 with c-kit staining of stem cells, or with periodic acid-schiff (PAS) staining to distinguish myocytes from conductive cells. In the sheep, the large conductive cells of the Purkinjie fiber system are localized predominantly in the subendocardial layers and have a different pattern of PAS staining from the cardiomyocytes. Our analysis of sections from fetuses of control and cortisol-treated ewes indicate caspase staining in at least 4 cell types: 1) cardiomyocytes; 2) diffusely scattered c-kit positive cells; 3) cells without PAS staining with fibroblast morphology, localized to collagen-abundant regions between myocyte bundles or in the subendocardial layer; and 4) large conductive cells with PAS-abundant staining localized in bundles within the subendocardial layer and projecting into the ventricular wall. In the cortisol-treated fetuses the latter cells, with Pukinjie characteristics, are the predominant cell type with caspase-3 staining in the subendocardial layer. These results suggest that in the preterm fetus, cortisol alters remodeling of the ventricular wall and of the developing conduction system. These results have implications for fetal and neonatal cardiac function and survival in fetuses of chronically stressed mothers.

T-121

Umbilical Cord Serum Insulin-Like Growth Factor Binding Protein-4 Expression Is Decreased in Normoglycemic Obese Pregnant Women at Term. Zachary M Ferraro,[1,2] Qing Qiu,[3] Andree Gruslin,[3,4] Kristi B Adamo.[1,2] *[1]Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Ottawa, Canada; [2]Healthy Active Living and Obesity Research Group, Children's Hospital of Eastern Ontario, Ottawa, Canada; [3]Ottawa Hospital Research Institute, The Ottawa Hospital, Ottawa, Canada; [4]Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Ottawa/The Ottawa Hospital, Ottawa, Canada.*

Rationale: Adipocyte hypertrophy and enhanced cellular proliferation resulting from energy imbalance is characteristic of weight gain. Epidemiological evidence suggests that maternal obesity is a risk factor for downstream child adiposity however the underlying physiological mechanisms responsible for this relationship are poorly understood. The insulin-like growth factor (IGF) axis has been hypothesized as a candidate pathway given its neoplastic role in fetal adipose tissue growth and development in late pregnancy. Further, enhanced bio-stability and -availability of IGF-1 and its binding proteins (IGFBPs) within fetal circulation have been postulated to contribute to excessive fetal growth common in maternal obesity. Nevertheless, the effect maternal obesity has on fetal IGF-1, IGF-2 and IGFBP expression, growth and development remains incomplete. Objective: We examined fasting serum samples obtained from 12 lean and 12 obese normoglycemic women (BMI= 30.61 ±11.76) undergoing elective caesarean section and the corresponding umbilical venous serum to test the hypothesis that maternal obesity, independent of impaired glycemia, is associated with up-regulated growth factor expression in the fetus. Methods: Using a western blot to detect mature IGF-1, -2 and western-ligand blot for IGFBP-1, -3 and -4 we aimed to quantify IGFs in maternal and fetal circulation at term. Results: Contrary to our hypothesis, neither mature IGF-1, IGF-2, IGFBP-1or IGFBP-3 (P>0.05) expression in maternal and fetal circulation did not differ between lean and obese mothers. However, IGFBP-4 expression was significantly depressed in cord serum in maternal obesity (P=0.04). Conclusion: Our results suggest that IGF bioavailability may play a significant role in the excessive fetal growth characteristic of maternal obesity. Future studies should examine other maternal and placental factors that may be implicated in the intergenerational transmission of obesity.

T-122

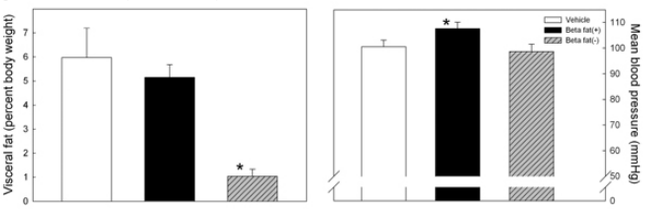
Antenatal Betamethasone Effects on Visceral Fat Function Are Associated with Cardiometabolic Programming in Adult Sheep. Jorge P Figueroa, Jie Zhang, Angela G Massman. *Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, USA.*

Glucocorticoid (GC) exposure in the perinatal period is associated with hypertension and alterations in the renin-angiotensin system (RAS) in brain and kidney in adult life. Furthermore, antenatal exposure to GC is thought to alter adipose (AT) tissue development. Adipose tissue RAS contributes to obesity-associated hypertension, but the mechanisms are not completely understood. The aim of this study is to increase our understanding on the role of visceral fat in the development of hypertension following antenatal GC exposure.

OBJECTIVE: To determine 1) if visceral fat removal ameliorates the effects of antenatal glucocorticoid exposure on the observed cardiometabolic abnormalities.

METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BETA, 0.17 mg/kg) 24-h apart at 80 days gestational age and allowed to deliver at term. At 2 mo of age, a subset of lambs (5) underwent surgical removal of the greater and lesser omenta and perirenal fat. Intact animals Beta treated (5) and Vehicle treated (5) served as control. Animals were studied at 6 and 12 months of age. Sheep were chronically instrumented under general anesthesia to place intravascular catheters. Insulin sensitivity was evaluated by iv glucose tolerance test (IVGTT). Data Mean±SEM were analyzed by ANOVA and/or two sample t test.

RESULTS: Surgical removal of AT resulted in a significant reduction of visceral AT both at 6 and 12 months (Left Panel) without significant effects on body weight Vehicle: 73±4.9, Beta fat(+): 82±4.6 and Beta fat(-): 83±7.9 (Kg). As shown on Right Panel, AT removal normalized mean blood pressure in Beta exposed sheep and also improved the insulin response to an intravenous glucose bolus (not shown).



CONCLUSION: Our data show that prenatal exposure to GC at 0.55 gestation has long-term effects on visceral AT function. Further studies are required to identify the intimate mechanism by which visceral AT affects both blood pressure control and insulin sensitivity. Based on these finding we propose that activation of AT RAS is one of the most likely mechanisms. HL 68728 and HD 047584.

T-123

SIRT1 Mediated Epigenetic Mechanisms Contribute to Programmed Fatty Liver in IUGR Offspring. Ming Gong, Diana Wolfe, Michael G Ross, Mina Desai. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

OBJECTIVE: Intrauterine growth restricted newborns (IUGR) have increased risk of adult metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). In rats, maternal food restriction during pregnancy results in IUGR newborns, which when nursed normally develop adult obesity and NAFLD. Notably, fatty liver is evident in IUGR fetuses (e20), suggesting a gestationally programmed effect. SIRT1, a nutrient sensor and histone deacetylase, epigenetically regulates hepatic lipid homeostasis by inhibiting expression of lipogenic factors. We hypothesized that reduced SIRT1 activity contributes to NAFLD in IUGR offspring by activation of lipogenic factors. We further evaluated the mechanism for altered SIRT1 expression, examining SIRT1 promoter methylation and histone H3 trimethylation (H3K9) status in fetal, newborn and adult IUGR and Control male offspring.

METHODS: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. All pups were nursed by control dams and weaned to standard rat chow diet. On embryonic day 20 (e20), postnatal age (p1) and at 3 months of age, livers were collected. SIRT1 protein expression (Western blot) and deacetylase activity, and methylation status of SIRT1 promoter (bisulfite genomic sequencing), and H3K9 (chromatin immunoprecipitation–qPCR) were determined, in conjunction with measures of hepatic triglyceride content and expression of lipogenic factors (SREBP1 and fatty acid synthase).

RESULTS: In IUGR offspring, SIRT1 expression was elevated at all ages (e20, 2.7-fold; p1, 1.4-fold; and 3 months, 1.9-fold) with parallel changes in SIRT1 activity as compared to Controls. Consistent with this finding, IUGR hepatic triglyceride content, SREBP and fatty acid synthase showed increased expression. SIRT1 promoter methylation status and H3K9 methylation was decreased.

CONCLUSION: Increased hepatic SIRT1 expression and activity prior to development of obesity suggests that SIRT1 mediated epigenetic mechanisms likely contribute to programmed NAFLD liver in IUGR offspring. Nutrient-induced upstream epigenetic processes regulate SIRT1 expression.

T-124

GnRH Analogues Decrease Leiomyoma Size by Directly Inhibiting Extracellular Matrix Production. William H Catherino,[1,2] Joy Britten,[1] Minnie Malik.[1] *[1]Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [2]Program in Reproductive and Adult Endocrinology, National Institute of Child Health and Human Development, Bethesda, MD, USA.*

Background: Uterine leiomyomas are highly prevalent tumors made up predominantly of extracellular matrix (ECM). Gonadotropin releasing hormone (GnRH) analogues have proven efficacy in decreasing leiomyoma size. The proposed mechanism of action requires a hypoestrogenic state, which results in significant symptoms including hot flashes and bone loss. Recent data suggests that (1) leiomyomas express both GnRH and GnRH receptors and (2) GnRH-treated leiomyomas differ from untreated leiomyomas in their molecular phenotype. Since GnRH analogues have a longer half-life than the native protein and are provided systemically, these compounds may directly impact leiomyoma cells.

Hypothesis: GnRH analogues decrease leiomyoma growth by inhibiting proliferation, ECM formation, and proteoglycan synthesis.

Materials and Methods: Human uterine leiomyoma and patient-matched myometrium were immortalized under an IRB-approved protocol and treated with leuprolide cetrorelix acetate. Proliferation studies and RNA/protein analysis were performed. All studies were done in the absence of gonadal hormones.

Results: Neither leuprolide nor cetrorelix influenced leiomyoma cell proliferation. Leuprolide acetate demonstrated a 'flare-effect' on ECM production, most notably at 6 hours treatment, which abated by 120 hours. Indeed, cetrorelix also demonstrated subtle agonistic properties at short time courses and low concentrations. GnRH analogue treatment resulted in decreased mRNA and protein expression of GnRH receptor 1 (2-fold), COL1A1 (20-fold), and fibronectin (6-fold) at therapeutic concentrations. The proteoglycan versican mRNA and protein expression was also decreased 3-fold by GnRH analogue therapy. Decreases in expression demonstrated both a time- and concentration-dependent effect. Dermatopontin expression increased 2-fold after 5 days leuprolide treatment.

Conclusions: GnRH analogues decrease leiomyoma size by inhibiting proteoglycan expression and decreasing ECM protein production. They do not inhibit cell proliferation. These findings demonstrate (1) GnRH analogues act directly on the leiomyoma, thereby providing the possibility of new delivery systems that minimize or prevent systemic symptoms, and (2) effective leiomyoma therapies regulate ECM rather than proliferation.

T-125

Treatment with CDB-2914 Altered AKAP13 Expression in Uterine Fibroids. Sydney Chang, Qingxiang Wei, Lynnette K Nieman, Alan H DeCherney, James H Segars. *Program in Reproductive and Adult Endocrinology, NICHD, NIH, Bethesda, MD, USA.*

Background: Uterine fibroids are stiff tumors that are dependent, in part, on sex steroids for growth. Treatment with anti-progestins has been shown to reduce fibroid size, but the mechanism responsible for the reduction remains unclear. The Rho-guanine nucleotide exchange factor (Rho-GEF) AKAP13 is highly expressed in fibroids, is involved in mechanical signaling, and may contribute to the increased stiffness and growth of these tumors; however, it is unknown whether anti-progestins affect the AKAP13/RhoA signaling pathway. The objective of this study was to determine whether AKAP13 expression, or localization, was altered by treatment with the anti-progestin, CDB-2914.

Materials and Methods: Fibroid and patient-matched myometrial tissue was collected from women treated with CDB-2914 (10 mg or 20 mg) or placebo for 3 months under an IRB-approved clinical protocol at the NIH. Immunohistochemical analysis was performed on specimens from patients in each of the 3 treatment groups (placebo, 10 mg, and 20 mg) using antisera directed against AKAP13. Intensity scores for nuclear, cytoplasmic, and overall staining were compared between fibroids of different treatment groups, myometrium of different treatment groups, and fibroids and myometrium within each of the treatment groups. Statistical analysis was performed using a Student's t-test.

Results: In general, AKAP13 expression was reduced in CDB-2914-treated fibroids, and increased in CDB-2914-treated myometrial specimens. In the 10 mg CDB-2914 treatment group, there was a significant decrease in cytoplasmic and nuclear staining in fibroids compared to myometrium ($p<0.05$). In the 20 mg treatment group, there was also a decrease in nuclear and overall staining in fibroids compared to myometrium. Finally, there was an increase in nuclear staining in the 10 mg CDB-2914-treated myometrial tissue compared to placebo-treated myometrium.

Conclusion: These results suggest that treatment with the anti-progestin, CDB-2914, altered spatiotemporal localization and expression of AKAP13 in fibroids. If confirmed with other approaches, these results suggest anti-progestin treatment may be associated with an alteration in Rho-mediated mechanical signaling.

This research year was made possible through the Clinical Research Training Program, a public-private partnership supported jointly by the NIH and Pfizer Inc (via a grant to the Foundation for NIH from Pfizer Inc).

T-126

MiR-106b/93 Represses Leiomyoma Cellular Proliferation by Direct Targeting of Cyclin D1 and E2F1 and Indirect Regulation of MCM7 Expression. Tsai-Der Chuang, Nasser Chegini. *OB-GYN, UF, Gainesville, FL, USA.*

It is estimated that 20 to 30% of leiomyomas are affected by non-random chromosomal abnormalities, including rearrangements and/or deletion of chromosome 7q22-q32, resulting in gain- or loss-of function of a large number of genes which are considered to play a key role in genesis of leiomyoma. Chromosome 7q22 harbors the intronic miR-106b~25 cluster and its host gene MCM7 with central role in cell cycle progression which is regulated by E2F1 and CCND1 in G(1) to S phase transition. Here we investigated the regulatory function of MCM7, miR-106b/93 and their target genes, E2F1 and CCND1, in genesis of leiomyomas. Using quantitative real-time PCR we demonstrated the expression of miR-106b/93 and MCM7 in leiomyoma and paired myometrium (N=63) and found that 60.98% (25/41) of leiomyomas expressed lower levels of miR-93 ($p=0.04$) and 65% (26/40) expressed elevated levels of MCM7 ($p=0.0225$) with no significant difference in miR-106b expression ($p=0.3394$; 48.78% or 20/41) as compared to myometrium. Western blot analysis indicated that MCM7 as well as CCND1 and E2F1 are expressed at elevated levels in leiomyomas and gain-of function of miR-106b/93 in isolated myometrium and leiomyoma smooth muscle cells (MSMC and LSMC) down-regulated the expression of E2F1 and CCND1 through direct interaction with their 3'UTR and inhibited the rate of cell proliferation. Gain-of-function of miR-93, but not miR-106b, also reduced the expression of MCM7 which was indirect and mediated through repression of E2F1 expression. In conclusion we demonstrated that miR-106b/93 and MCM7 are co-expressed and functionally regulate the rate of cell proliferation by targeting the expression of CCND1 and E2F1, and by miR-93 regulation of MCM7 expression through feedback inhibition of E2F1. We propose that the consequence of chromosomal re-arrangement affecting ch7q22 resulting in changes in the expression and/or regulation of miR-106b/93 and their downstream target genes may influence leiomyoma cellular proliferation and their subsequent growth (Supported by NIH grants HD37432 and HD58664).

T-127

Effects of Ulipristal Acetate, a Selective Progesterone Receptor Modulator, on Activin and Myostatin System Expression and Function in Uterine Leiomyoma. Pasquapina Ciarmela,[1] Patrizia Carrarelli,[2] Md Soriful Islam,[1] Claudia Tosti,[2] Mario Castellucci,[1] Felice Petraglia.[2] *[1]Experimental and Clinical Medicine, Polytechnic University of Marche, Ancona, AN, Italy; [2]Pedriatics, Obstetrics and Reproductive Medicine, University of Siena, Siena, SI, Italy.*

Selective progesterone modulators (SPRMs) belong to a new class of drugs which have the characteristics to show different action in different tissues, exhibiting agonistic or antagonistic properties in a cell and tissue context dependent manner; they also provide the therapeutic effects of progestins while avoiding their side effects. One of the possible clinical implication is the medical treatment of uterine leiomyoma.

Randomized clinical trials are showing that SPRM ulipristal acetate (UPA also know as CDB-2914) reduces leiomyoma size and symptoms. However, an understanding of the molecular mechanisms of UPA action is of paramount importance for the development of the therapeutic strategies. Uterine leiomyoma express both estrogen and progesterone receptors and are considered to be hormone dependent benign tumors. The action of sex steroids hormones in their target tissues is considered to be mediated by local production of growth factors, locally acting through paracrine and/or autocrine mechanisms. Activin βA (INHBA) and myostatin are growth factors recently identified in myometrial cells and were shown to be steroid hormone dependent modulators of myometrial cell proliferation. Activin-A and myostatin expression levels

are higher in leiomyoma compared to adjacent myometrium explants, but do not trigger a transcriptional response in this tissue. Hence, disruption of their signaling may contribute to fibroid growth.
The aim of the present study was to evaluate the effects of UPA on activin and myostatin system expression and function in myometrium, leiomyoma and endometrial tissues.
Tissues collected from uterine leiomyoma, adjacent normal myometrium and endometrium were cultured and treated for 24h with increasing doses of UPA (10^{-8}, 10^{-7}, 10^{-6} M). Expression level of activin βA, myostatin and molecules involved in their signaling such us their receptors (ActRII, ActRIIB, ALK4, ALK5) and binding proteins (follistatin, FLRG, cripto) were evaluated by real-time PCR.
The proposed study improves the understanding of UPA mechanism of action.
Acknowledgements: The present study was supported by PregLem (Geneva, Switzerland).

T-128

Uterine Leiomyoma Growth Modeled through Molecular Networks. Barbara J Davis,[1] John I Risinger,[2] Gadisetti VR Chandramouli,[2] Pierre Bushel,[3] Donna Day Baird,[4] Shyamal Peddada.[3] *[1]Biomedical Sciences, Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA; [2]Obstetrics Gynecology and Reproductive Biology, College of Human Medicine Michigan State University, Grand Rapids, MI, USA; [3]Biostatistics Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC, USA; [4]Epidemiology Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC, USA.*
The underlying molecular characteristics related to the growth of uterine leiomyoma are largely unknown. Previous analysis of uterine leiomyomata (fibroids) in a clinical study revealed differential subsets of tumors with distinguishing growth characteristics. We compared gene expression patterns between these different groups of fibroids with the goal to identify genes most closely associated with mechanisms of growth. A total of 52 fibroids from black and white women of different ages and 8 myometrial samples were analyzed using Affymetrix Human Genome U133A plus 2.0 microarray technology and Microarray Suite 5.0 software. Comparisons were then made between all fibroids to myometrium and between fibroids likely to be rapidly growing to fibroids likely to be slow- or non-growing. Genes that were declared to be significant in a pairwise comparison were further analyzed for canonical pathways, networks and biological functions using the Ingenuity Pathway Analysis software. Whereas our comparison of leiomyoma to myometrium produced a very large list of genes highly similar to numerous previous studies, distinct sets of genes and signaling pathways were identified in comparisons of fibroids with likely different rates of growth. Significant gene-related biological functions identified in growing vs. non-growing fibroids related to lipid metabolism; canonical pathways identified included retinoic acid, hypoxia signaling, and mitochondrial dysfunction; and the most significant networks centered on the up-regulation of VHL and the down-regulation of EGFR. These findings serve as the basis for a molecular model of fibroid growth that centers on alterations in metabolism and response to hypoxia.

T-129

T2-Weighted Signal Intensity Correlates with Histology in Patients with Hereditary Leiomyomatosis and Renal Cell Cancer Syndrome. Kate Devine,[1] Armstrong Y Alicia,[1] James H Segars,[1] Merino Maria,[2] W. Marston Linehan,[2] Stratton Pamela,[1] Venkatesan Aradhana.[3] *[1]Program in Reproductive Endocrinology and infertility, Eunice Kennedy Shriver National Institutes of Child Health and Human Development, National Institutes of Health; [2]National Cancer Institute, National Institutes of Health; [3]Clinical Center, National Institutes of Health.*
Objective: Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) is an autosomal dominant syndrome characterized by susceptibility to renal cell cancer, skin and cellular uterine leiomyomas, and possibly, an increased incidence of leiomyosarcoma. An association between cellular fibroids and increased T2-weighted MRI signal intensity has been suggested, compared to the characteristically low signal intensity of common fibroids. We sought to delineate the association between high signal intensity on MRI and histopathologic characteristics of HLRCC-associated leiomyomas.
Methods: Five women with a confirmed diagnosis of HLRCC underwent pelvic MRI prior to hysterectomy or myomectomy. T2-weighted MRI signal intensity of leiomyomas was characterized as described (Funaki et al. 2006): (1) low, similar to muscle (2) intermediate, between skeletal muscle and myometrium (3) high, greater than or equal to myometrium. Representative samples of leiomyoma(s) from each case were sectioned, and standard H&E staining and microscopic evaluation was performed by a single gynecologic pathologist. A single, interpreting radiologist analyzed all MRIs and was blinded to pathologic diagnosis.
Results: 43 fibroids were characterized as Funaki 2/3 by T2-weighted MRI, which comprised the majority of fibroids overall, as well as for each individual patient. Three patients underwent myomectomy, and two underwent hysterectomy. 26 of 26 fibroids sectioned (100%) were classified as cellular/atypical. Specifically, the specimens demonstrated increased cellularity, nuclear pleomorphism, and occasional mitotic figures. 7 of 26 fibroids from 2 patients were subcategorized as smooth muscle tumor of undetermined malignant potential. No cases of leiomyosarcoma or any malignancy were diagnosed.
Discussion: MRI T2 signal intensity in all patients was higher than observed in common, spontaneous fibroids, and all leiomyomas studied were assessed as atypical by surgical pathology. The results of this pilot study support a larger study to determine if MRI may enable surveillance of leiomyoma in HLRCC patients without surgical intervention.

T-130

Vitamin D3 Suppressed ER-α Gene Expression Via Regulation of Steroid Receptor Co-Activators in Uterine Leiomyoma Cells. Sunil K Halder,[1] Kevin G Osteen,[2] Ayman Al-Hendy.[1] *[1]Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, TN, USA.*
Background: Uterine leiomyomas (fibroids) are the most common estrogen-dependent benign tumors. Black women bear three- to four times higher risk of uterine leiomyomas and ten times more prevalence in hypovitaminosis D than white women. Previously, we showed that vitamin D3 reduced estrogen receptor-alpha (ER-α) in a dose-dependent manner in human uterine leiomyoma (HuLM) cells. Currently, it is not well understood how vitamin D3 regulates ER-α gene expression in uterine leiomyomas.
Objective: Leiomyoma tumors are known to be regulated by estrogen. However, the role of steroid receptor coactivator (SRC) family members in the growth of fibroid is currently unknown. Herein, we will compare the expression levels of SRC-1, SRC-2, and SRC-3 between fibroids and adjacent normal myometrium, and to evaluate the effect of vitamin D3 on these SRCs in the HuLM cells.
Design: Paired uterine fibroids and normal adjacent myometrium tissue samples were homogenized and sonicated briefly in cell-lysis buffer to prepare clear protein lysates. Protein lysates were also prepared from HuLM cells after vitamin D3 treatment for 48 hours and were analyzed by western blotting. Immunofluorescence analyses were also performed for subcellular localization of ER-α, SRC-1, SRC-2, and SRC-3 in HuLM cells.
Results: The expression levels of SRC-2 and SRC-3 were higher in fibroids (at least 50% cases) when compared to adjacent normal myometrium tissues. However, the expression levels of SRC-1 were not changed significantly in the fibroids. Furthermore, we found that vitamin D3 decreased the expression levels of SRC-1, SRC-2, and SRC-3 in a dose-dependent manner in HuLM cells ($p<0.05$). Additional analyses by immunofluorescence showed reduced expression of nuclear SRC family proteins in vitamin D3-treated HuLM cells. These results demonstrate that vitamin D3 can reduce uterine leiomyoma growth via the regulation of steroid receptor coactivators.
Conclusions: Vitamin D3 is a potent regulator of SRC family proteins, and thus could impart its actions at least partially through steroid receptor signaling. Vitamin D3 could potentially be useful as safe and non-surgical therapeutics for the treatment of uterine leiomyomas.
Support: RCMI pilot 2G12RR003032-26, CRC-MeTRC pilot 202142-535001-20, and NIH/NICHD R01 HD046228.

T-131

Vitamin D3 Inhibits Uterine Leiomyoma Growth Via Down-Regulation of MMP-2 and MMP-9 Activities. Sunil K Halder,[1] Kevin G Osteen,[2] Ayman Al-Hendy.[1] *[1]Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, TN, USA.*
Background: Uterine leiomyomas (fibroids) are benign tumors that result from the dysregulated proliferation of normal myometrial smooth muscle cells (SMCs) and tissue reorganization/remodeling. The pathogenesis of uterine leiomyoma is unknown and most uterine fibroids are asymptomatic, their growth can attribute to many discomforts such as miscarriage, infertility, menstrual problems, excessive bleeding, preterm labor and recurrent abortion. Black women are at four times greater risk than white women for developing uterine leiomyomas. However, the reasons behind this disparity are still unknown.

Objective: The extracellular matrix (ECM) plays an important role in tissue remodeling which is a key process in the growth of uterine leiomyomas. Herein, we examined the expression levels and activities of matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9 that play a role in the degradation of ECM. Design: Immortalized human uterine leiomyoma (HuLM) cell line and primary uterine leiomyoma (LM) cells were used to elucidate the expression levels of several MMPs as well as enzyme activities of MMP-2 and MMP-9, with and without vitamin D3 treatment. Lysates from vitamin D3-treated and untreated control cells were used to examine the expression levels of several MMPs by western blotting using specific antibodies. Supernatants from culture mediums were also analyzed using gelatin zymography to detect the activity levels of both MMP-2 and MMP-9.
Results: Our results showed that protein levels of both MMP-2 and MMP-9 were decreased by vitamin D3 in a dose-dependent manner ($p<0.05$). The pro-MMP-9 was down-regulated by vitamin D3 in a dose-dependent manner while the active MMP-9 was undetectable. Both the active and pro- forms of MMP-2 were detectable, but were down-regulated by vitamin D3 in a dose-dependent manner ($p<0.05$). Additionally, the inhibitor of MMPs, TIMP-2 was induced by vitamin D3 treatment in both cell lines. However, the levels of other MMPs including MMP-1, MMP-10, and MMP-13 were not changed significantly by vitamin D3.
Conclusions: Vitamin D3 reduces the expression and activities of MMP-2 and -9 while inducing the TIMP-2 which may be important in inhibiting the growth of uterine leiomyomas.
Support: RCMI pilot 2G12RR003032-26, CRC-MeTRC pilot 202142-535001-20, and NIH/NICHD R01 HD046228.

T-132

Towards Gene Therapy for Uterine Fibroids: Enhanced Efficacy of Fibroid-ON/Normal Tissue-OFF Adenovirus Vector Ad-SSTR-RGD-TK. Sangeeta Nair,[1] David Curiel,[2] Ayman Al-Hendy.[1] *[1]CWHR, Obstetrics Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Radiation Oncology-Cancer Biology, Washington University, St Louis, MO, USA.*
Background: Uterine fibroids are common gynecological tumors in premenopausal women. Treatment options include gene therapy-a potentially effective, non-invasive localized method.We have demonstrated that adenoviral (Ad) vectors optimally transduce rat uterine leiomyoma cells and reduce leiomyoma volume significantly (Gynecol Obstet Invest. 2009; 68:19-32). We also reported that targeted Ad modified by adding an RGD (arginine-glycine-aspartic acid) motif, is coxsackie adenovirus receptor independent possessing increased transduction affinity to human leiomyoma cells.
Objective: To test the efficacy of Ad-SSTR-RGD-TK vector expressing thymidine kinase (TK)/ganciclovir (GCV) therapeutic gene system against immortalized human uterine leiomyoma cells (HuLM) and smooth muscle cells (UtSMC). An imaging cassette (SSTR) is added to the vector to facilitate future in vivo tracking.
Methods: HuLM and UtSMC were transduced with Ad vectors: Ad-Lac Z (β-galactosidase), unmodified first generation Ad-TK or targeted Ad-SSTR-RGD-TK at 5-100 plaque forming units [pfu]/cell,and GCV at10µg/ml. Cell growth was measured on day 4 using CyQuant assay (Invitrogen).Western blot was performed on Ad transduced HuLM and UtSM cell lysates for detecting PCNA, Cyclin D-1, BCL-2 and BAX protein. Statistical analysis was done using Student's t-test.
Results:Cell count in HuLM cells transduced with Ad-SSTR-RGD-TK at 5,10 and 50 pfu was reduced by 27,40 and 77% respectively compared to Ad-LacZ ($P<0.05$). Western analysis of HuLM cells transduced with Ad-SSTR-RGD-TK showed a dose dependent decrease in Cyclin-D1 and PCNA and increase in proapoptotic BAX protein. In UtSMC transduced with Ad-SSTR-RGD-TK, cell count reduced by 10,23 and 25% compared to Ad-LacZ ($P<0.05$). Western analysis of UtSMC transduced with Ad-SSTR-RGD-TK and Ad-Lac Z showed no difference in the expression of BAX, BCL-2 and PCNA. Ad-SSTR-RGD-TK consistently demonstrated superior transduction of HuLM cells compared to Ad-TK.
Conclusions: Ad-SSTR-RGD-TK/GCV effectively reduces growth in HuLM cells compared to UtSMC appearing as "fibroid-ON/normal myometrium-OFF" switch. These data demonstrate the potential utility of Ad-SSTR-RGD-TK/GCV gene therapy approach as a viable treatment option for symptomatic uterine fibroids.
Support: NIH/NICHD R01 HD046228 and RCMI grant G12 RR 03032

T-133

Safety Profile and Patient Experience Following Magnetic Guided Focused Ultrasound in the Treatment of Uterine Fibroids. Stephen D Quinn,[1] Vedelago John,[2] Casey Murrey,[2] Wladyslaw M Gedroyc,[2] Lesley A Regan.[1] *[1]Obstetrics and Gynaecology, Imperial College London, London, United Kingdom; [2]Radiology, Imperial College London, London, United Kingdom.*
Introduction: Magnetic resonance guided focussed ultrasound (MRgFUS) ablation of uterine fibroid tumours has emerged as an alternative to traditional forms of fibroid therapy. In this study we reviewed the safety of this treatment modality at St Mary's Hospital, London, UK.
Methods: Retrospective review of 281 women undergoing MRgFUS between 2003 and 2010. Data was obtained from patient records and patient questionnaires. In addition we undertook telephone consultations on all women during which a standardized questionnaire was completed to assess their personal experience of the treatment and outcomes.
Results: Notes and patient records were reviewed for all women, and we successfully contacted 223 of the 281 0(79%) for telephone consultation. No adverse events and no significant pain were reported in 249 of the 281 women (87%). Mild to moderate pain, lasting up to 5 days post procedure was reported by 18 of 281 women (6%) in the absence of any other significant complications. Minor complications (urinary tract infection, retention, vaginal bleeding, transient buttock pain) were experienced by 11 of 281 women (4%). Only 3 women (1% of the total cohort) experienced severe complications. These included one case of fibroid expulsion, one major skin burn requiring surgical repair, and one case of persistent neuropathy. No emergency hysterectomies were required following MRgFUS therapy. One woman required hospital admission for urinary retention, which resolved after 3 days.
Conclusions: In comparison to laparoscopic or open myomectomy and uterine artery embolisation, MRgFUS ablation procedures are associated with less serious complications, and adverse events occur less frequently. With the recent development of improved real time imaging and enhanced 3-dimensional ultrasound probe direction control, the favourable safety record of magnetic resonance-guided focussed ultrasound can only improve further.

T-134

Predictors of Morbidity in Women Undergoing Abdominal Myomectomy. Samantha B Schon,[1] Cooper R Amber,[2] Valerie S Ratts,[2] Odem R Randall,[2] Sarah L Keller,[2] Emily S Jungheim.[2] *[1]OB/GYN, Washington University in St. Louis, Saint Louis, MO, USA; [2]REI, Washington University in St. Louis, Saint Louis, MO, USA.*
Background and Objective: Abdominal myomectomy has long been the approach to large myoma burdens in women seeking fertility treatment, but this procedure can be associated with significant morbidity. As a result, myoma treatment options associated with less morbidity are emerging. The objective of this study was to determine predictors of morbidity in abdominal myomectomy as knowledge of these predictors may aid in weighing options for treating myomas.
Methods: Participants were identified through hospital billing records and excluded if myomectomy occurred with other procedures. Standard univariate analyses were used to identify predictors associated with adverse surgical outcomes including: operative (OR) time, blood loss, transfusion, hospital stay ≥4days and residual fibroids. Multivariable regression models were built to determine which predictors remained significant after controlling for potential confounders. Receiver-Operator Characteristic curves were used to establish if a specific uterine volume was sensitive and specific for predicting adverse outcomes. $P<0.05$ was considered significant.
Results: 90 women met study criteria. Predictors associated with adverse outcomes in univariate analyses included: BMI≥25, African American race, large uterine volume (LUV) and prior myomectomy. In multivariate analyses BMI≥25 and LUV were associated with longer OR time. LUV and prior myomectomy were associated with increased blood loss. LUV was associated with transfusion and longer hospital stay. BMI≥25 was associated with residual fibroids after surgery. Race was no longer significant after controlling for potential confounders. Uterine volume ≥1259cm^3 was sensitive and specific for transfusion(82%,74%) and longer hospital stay(65%,68%).
Conclusions: BMI≥25, large uterine volume or prior myomectomy are associated with morbidity in abdominal myomectomy. In addition, uterine volume ≥1259cm^3 was associated with transfusion and longer hospital stay. More work is needed to establish if these predictors help in deciding if other fibroid treatment modalities are preferable to abdominal myomectomy for some due to surgical risks. This work may include decision and cost effective analyses that include individualized treatment goals and risk profile.

T-135

Sharan 1: A Novel Mouse Model for Human Uterine Fibroids. Chakradhari Sharan, Sunil K Halder, Sangeeta Nair, Ayman Al-Hendy. *Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*
Background: Uterine fibroids are the most common gynecological tumors that are associated with infertility, menorrhagia and spontaneous abortion. High frequency of these tumors in the population, especially in African American women makes uterine fibroid a significant reproductive health hazardous for women. The etiology of uterine fibroid is not fully understood. To study these tumors, we need simple and easily available, but reliable animal model system. Currently, only two animal models—the Eker rats and Memy-1 (developed in our lab) are available. Eker rats develop uterine fibroids by 12-16 months of age with a frequency of 65% or less. And, Memy-1 model requires a suitable and fully committed donor for the tissue to be xenografted. The processing of the tissue before implantation in animal model is also tedious and time consuming. Herein, we used an immortalized human uterine leiomyoma (HuLM) cell line to generate subcutaneous tumors in the NOG mice.
Objective: To develop a simple and reliable mouse model for human uterine fibroids. Design: Immune deficient NOG mice were injected with $15x10^6$ HuLM cells subcutaneously (SC) in the dorsal side of pelvic region supplemented with SC implantation of 50 mg progesterone and 1.7 mg estrogen 90-day slow-release pellet. All experimental animals were divided into 6 groups: Control group 1) received only HuLM cells; 2) received estrogen pellet along with the cells; 3) received progesterone pellet along with the cells; 4) received both progesterone and estrogen along with the cells; 5) received immortalized rat uterine leiomyoma derived ELT3 cells (as a positive control) and 6) did not receive anything and served as a negative control.
Results: We observed that mice in group (4) receiving both estrogen and progesterone produced visible subcutaneous tumors within 45 days after HuLM cell implantation and these lesions continued to grow for 85 days. Tumor tissues analyses by H&E staining exhibited typical human fibroid histology. The proliferation marker, Ki67 exhibited more proliferating index whereas less apoptotic cells were detected by caspase-3 staining. Conclusion: These results suggest that both estrogen and progesterone are necessary for the develpment of human leiomyomas.
This mouse model (Sharan 1) can enhance research to develop safe and effective therapeutics for uterine fibroids.This research is supported by NIH/ NICHD RO1 HD046228.

T-136

Hypoxia Stimulates Endothelin-1 Secretion from Human Myometrium Explants; a Potential Link between Hypertension and Development of Uterine Leiomyomas. Kedra L Wallace, Justin Porter, Evan Turnage, Krystal Frazier, Venessia Johnson, Babbette LaMarca. *Ob/Gyn, University of MS Medical Center, Jackson, MS, USA.*
Background. Uterine leiomyoma (fibroids) are benign tumors of the myometrium and are the most common tumor of the female reproductive tract. Intermittent periods of hypoxia have been implicated as a possible mechanism by which myometrial cells could undergo aberrant cell growth and possible differentiation into leiomyomas. Endothelin-1 (ET-1), a potent vasoconstrictor peptide, is increased in fibroids in response to hypoxia and inhibits apoptosis of leiomyoma cells *in vitro*. We have previously shown a strong association between hypertension in our uterine leiomyoma patient population and ET-1 production as a result of hypoxia, suggesting a link between the development of uterine leiomyoma and hypertension. **Objective**. This study was designed to test the hypothesis that hypoxia stimulates ET-1 production in the human myometrium. **Methods.** Immediately after hysterectomy, fibroids and myometrium are excised from the uterus, washed and plated on matrigel coated 6 well inserts and cultured in standard media. To induce hypoxia, cultures were placed in a modular incubator with the final pO_2 value between 1-0.5% for 24hrs. A 6 hour period of intermittent hypoxia (IMH) was followed by 18hours of normoxia (final pO_2 6%). ET-1 was measured from cell culture supernatants using a Quantiglo ET-1 ELISA. Tissue mRNA was extracted, cDNA transcribed and real-time PCR utilized to measure preproendothelin-1 (ET-1) and GAPDH expression. **Results.** ET-1 was increased in fibroid media compared to media from myometrium cultures (9.69 ± 2.72 vs 6.81 ± 1.41pg/mg/mL). ET-1 mRNA levels were significantly increased in fibroid tissue (14.37 ± 0.11) compared to myometrium tissue ($10.28\pm.56$; $p<0.002$). After 24hrs of hypoxia ET-1 secretion increased from the myometrium (8.10 ± 2.38pg/mg/mL) to levels comparable to ET-1 secretion from normoxic fibroid cultures ($p=0.716$). 2 and 4 periods of IMH in the myometrium increased ET-1 mRNA expression compared to non-exposed fibroid tissue (13.27 vs 8.42). **Conclusions.** These data indicate that ET-1 is increased in the fibroid and fibroid ET-1 secretion is stimulated by hypoxia. We further demonstrated that hypoxia stimulates ET-1 secretion from myometrium explants, similarly to what is seen in the fibroid, thereby suggesting a possible link between hypoxic stimulated ET-1 and hypertension in uterine leiomyoma patients.

T-137

Analysis of the Genetic Expression of Neurotrophins in the Endometrium of Patients with and without Endometriosis. Hector Barrera-Villa Zevallos,[1,4] Donna Lai,[2] Jonathan Arthur,[3] Cecilia Ng,[1] Robert Markham,[1] Ian S Fraser.[1] *[1]Department of Obstetrics, Gynaecology and Neonatology, Queen Elizabeth II Research Institute for Mothers and Infants, The University of Sydney; [2]The Bosch Institute, The University of Sydney; [3]Children's Medical Research Institute, The University of Sydney; [4]National Council of Science and Technology, CONACyT.*
Background
Based on the high incidence of endometriosis observed amongst direct family members of women with the disease (RR = 7.2), is clear a genetic predisposition for endometriosis. Given that nerve fiber density is higher in the endometrium of women with endometriosis, it is possible that neurotrophic molecules are differently expressed by endometrial cells amongst women with and without endometriosis.
Objectives
To illustrate the genic expression of the neurotrophins and their receptors in the endometrium of women with and without endometriosis, to identify possible expression patterns inducing nerve proliferation throughout the menstrual cycle.
Methods
Total RNA was extracted from frozen endometrial tissue biopsies of patients without and with endometriosis (confirmed by laparoscopy and pathology), at different stages of the menstrual cycle, for microarray (7 cases and 11 controls) and multiplexed polymerase chain reaction based analyses (20 cases and 20 controls).
Results
10 genes revealed ≥2-fold over-expression and 2 other genes ≥2-fold under-expression in the group of endometriosis cases compared to controls. However, these changes did not reach statistical significance at $p<0.05$ when corrected for multiple testing using the Benjamini-Hochberg method. The multiplex PCR preliminary data suggest possible up-regulation of NTRK1, NGF and NTF4, and down-regulation of NTF3 and NTRK3 in the proliferative phase in endometriosis cases compared to controls
Conclusions
The GeXP and microarray analysis yielded results suggesting no significant difference of expression in the neurotrophin gene expression profiles between endometriosis and controls. These results did not reach statistical significance, however the optimization of the primer plex molar concentration is still under development.

T-138

Microarray Analysis of Methylation in Placentas from CBAXDBA Pregnancies in Mice Points to a Novel Mechanism for Pregnancy Loss. Stephen Brown, Elizabeth Bonney, Brian Nielsen, Renju Raj, Lucia Brown. *Obstetrics, Gynecology and Reproductive Science, University of Vermont, Burlington, VT, USA.*
When female mice of the inbred strain, DBA/2 (DBA/2F) are mated with CBA/J males (CBA/JM), few fetal resorptions are noted. However, when the opposite mating is performed (CBA/JF X DBA/2M), up to 50% of pregnancies are resorbed by gestational day 12-14. Because of this, CBA/JF X DBA/2M matings have served as a model system for miscarriage for many years; however, there is no molecular understanding of the cause for fetal loss.
F1 embryos from CBA/JF X DBA/2M matings are genetically identical to F1 embryos from DBA/2F X CBA/JM matings, making it unlikely that genetic differences between embryos contribute to the differential rate of loss. Therefore, it is assumed that pregnancy loss is in some way mediated by the maternal environment. As an alternative hypothesis, we have considered the possibility that there may be parent of origin (imprinting) differences between the two classes of genetically identical embryos. In this model, parent of origin (epigenetic) differences (as opposed to maternal environment) would contribute to pregnancy failure.
To test this model, we performed a microarray-based analysis of methylation of placental DNA of E9.5 embryos at 16,000 sites on chromosome 7. We show that DBA/2F XCBA/JM (healthy) embryos have highly consistent methylation profiles, while CBA/JF XDBA/2M (loss-prone) embryos show a striking degree of variability of methylation at thousands of loci, reflecting a highly disordered

epigenome. This was assessed by determining the variance of signal intensity across 7 microarray data sets for all 16K loci. We then compared the mean variance of the two groups using a z test. In this comparison, z was significant at 10E-40, confirming a striking degree of variability of methylation in loss-prone pregnancies.

Given what is known about the establishment of methylation during embryogenesis, the methylation abnormalities that we see are likely to have been present since implantation, and this suggests that the abnormalities are intrinsic to the embryo. Alternatively, the CBA/J maternal environment may act to alter methylation long before any overt evidence of pregnancy failure. Future experiments will be aimed at understanding whether methylation abnormalities are truly intrinsic to the embryo or depend on the maternal environment.

T-139

Elucidation of microRNA-Gene Networks in the Fetal Sheep Heart. Laura A Cox,[1,2] Jeremy Glenn,[2] Kimberly D Spradling,[1] Mark J Nijland,[3] Roy Garcia,[1] Peter W Nathanielsz,[3] Stephen P Ford.[4] *[1]Dept. Genetics, Texas Biomedical Research Institute, San Antonio, TX, USA; [2]Dept Genetics, Southwest National Primate Research Center, San Antonio, TX, USA; [3]OB/GYN, UTHSCSA, San Antonio, TX, USA; [4]Dept. Animal Science, University of Wyoming - Laramie, Laramie, WY, USA.*

MicroRNAs (miRNAs) are emerging as important gene regulators, biomarkers and therapeutics for human diseases. Because some miRNAs are important for cell proliferation and differentiation, we predict miRNAs play a role in regulation of fetal heart development. Discovery of these miRNAs and gene networks they regulate will provide insights into genome, transcriptome and epigenome interactions fundamental to cardiac development.

METHODS: RNA was extracted from fetal sheep left ventricle samples (n=14), cDNA libraries prepared and subjected to cluster generation and high throughput sequencing (Illumina GAIIx). Paired-end reads were assembled, annotated and expression quantified using the Cufflinks suite of tools and stepwise comparative genomic methods with cow and human genome sequences. Pathway (GeneSifter) and network analyses (Ingenuity Pathway Analysis) were used to identify coordinated gene networks that correlate with specific miRNA expression profiles.

RESULTS: We identified and quantified 36,996 genes and splice variants and 193 miRNAs expressed in the fetal sheep heart transcriptome. Only three of the miRNAs play known roles in cardiac and/or muscle development. We identified 25 high probability miRNA-gene networks. Among these is a network with miRNA miR-1, known to function in muscle development, as central hub. These are networks with miRNAs miR-124 and miR-30 as hubs for genes related to cell growth and differentiation. Neither of these miRNAs or their networked genes have been linked to cardiac development.

DISCUSSION: We undertook a discovery approach to identify transcriptional networks central to heart development that may be regulated by epigenetic mechanisms. Deep sequencing methods followed by pathway and network analyses provided an unbiased view, independent of species specific reagents, of the sheep fetal heart. This study revealed coordinated gene networks and miRNAs in the fetal heart transcriptome not previously described as playing roles in this process. This is a first step in understanding normal fetal heart development at the molecular level– an essential step for recognizing the dysregulated process. HD 21350, INBRE P20RR016474

T-140

Prevalence of NPC1 Gene Variants in the Local Obstetric Population and Their Association to Obesity and Gestational Diabetes. Lesley de la Torre, Matthew C Brennan, William F Rayburn, David Jelinek, William S Garver. *Obstetrics and Gynecology/Maternal-Fetal Medicine, University of New Mexico, Albuquerque, NM, USA.*

A recent Genome-Wide Association study determined that the Niemann-Pick C1 (NPC1) gene is associated with early-onset and adult obesity in European populations. The following NPC1 gene variants (His215R, Ile642M, Ile858V) are suspected to be associated with the development of obesity. The objective of this study is to determine the prevalence of these different NPC1 gene variants in our obstetric population.

A prospective, single center study was conducted in which pregnant patients admitted to the Labor Delivery Unit were considered eligible for participation if they received prenatal care during their current pregnancy at our institution. A blood specimen from each patient was sequenced in the laboratory for gene variant identification of the NPC1 gene.

53 blood specimens were available for analysis. Obesity (BMI ≥ 30) was present in 60% of our study population. The frequency of NPC1 gene variants H215R, I642M, and I858V were 9.4%, 3.8%, and 35.9% respectively. Descriptive studies were used to illustrate demographic characteristics. The gene variant, I858V was the most frequent NPC1 gene variant noted in this population.

This is the first known study of its kind that has studied the pregnant population in relation to these gene variants that may be closely associated with the development of debilitating metabolic conditions, such as, diabetes and obesity. From the studied gene variants, I858V was the most prevalent variant seen in our obstetric population.

Demographic Characteristics

Characteristic	**Median**	**Minimum**	**Maximum**
Maternal Age	25	18	36
Gestational Age	39	34	41
BMI	31	20	58
Ethnicity	**Frequency (n)**		**Percent (%)**
Caucasian	13		24.5
Hispanic	35		66
American Indian	5		9.4
Diabetes	7		13.2
H215R variant	5		9.4
I642M variant	2		3.8
I858V variant	19		35.9

Frequencies of NPC1 variants listed above are those expressed in a homozygous manner in the studied population.

T-141

Krüppel-Like Transcription Factor 11 (KLF11) Epigenetically Mediates TGF-β Signaling in Endometrial Cells. Ravi P Gada, Zaid M Tabbaa, Ye Zheng, Raul Urrutia, Gwen A Lomberk, Gaurang S Daftary. *Epigenetics and Reproductive Diseases Laboratory, Mayo Clinic, Rochester, MN, USA.*

Objective: TGF-β signaling has been implicated in cardinal endometrial functions such as decidualization and menstruation. The underlying molecular mechanisms are however, not well defined. TGF-β up-regulates Sp-1, which in turn activates TGF-β receptor synthesis resulting in a positive feedback circuit. We show here that the Sp-1 like transcription factor KLF11 counterbalances this mechanism via a critical ying-yang paradigm that affects overall TBF-β signaling in endometrial cells.

Methods: Expression of KLF11 and target TGF-β signaling pathway molecules was assessed in Ishikawa and endometrial stromal cells by PCR and western blot. KLF11 expression in the endometrium was evaluated by immunohistochemistry. Molecular targets of the TGF-β signaling pathway were identified by microarray and pathway reconstruction. Q-PCR, ChIP and EMSA were used for target confirmation. Target-gene regulation was assessed by PCR and Luciferase Reporter Assay.

Results:

TGF-β is known to regulate expression of KLF11. Here we show that KLF11 is not only a target of TGF-β signaling, but additionally also mediates TGF-β signaling rapidly and effectively via a novel Smad independent mechanism. KLF11 down-regulated TGF-β signaling detected by decreasing Phospho-Smad2 levels. KLF11 repressed multiple targets in the TGF-β signaling cascade - TGFβ Receptors I and II, SMAD2, 3 and BMP2R, which are known to mediate decidualization of endometrial cells. Interestingly, KLF11 repressed these targets epigenetically via Sin3a/HDAC1 resulting in chromatin condensation on each target gene promoter. Repression was consequently reversed by KLF11 siRNA and a mutation in KLF11 that abrogated Sin3a binding. ChIP and EMSA confirmed direct KLF11 binding to the promoters of these genes.

Conclusion: This is the first study to show that the transcriptional factor KLF11 is a critical non-Smad mediator of endometrial TGFβ signaling. Endometrial implantation requires decidualization, which is mediated by progesterone and TGF-β signaling. By altering the expression of multiple targets in the signaling pathway, KLF11 can potentially alter endometrial TGF-β signaling thereby affecting endometrial decidualization and receptivity. We show here that regulation by KLF11 is epigenetic via histone deacetylation and thereby potentially amenable to therapeutic intervention.

T-142

Functional Silencing of the Tumor Supressor KLF11 in Human Endometrial Cancer Reveals Pathways Which Are Amenable to Pharmacological Manipulation. Ravi Gada, Gaurang Daftary, Adrienne Grzenda, Navtej Buttar, Sean Dowdy, John Schoolmeester, Gary Keeney, Gwen Lomberk, Raul Urrutia. *ECDL (Epigenetic and Chromatin Dynamics Laboratory) and ERDL (Epigenetic of Reproductive Diseases Laboratory), Department of Medicine, Obstetrics and Gynecology, Mayo Clinic, Rochester, MN, USA.*

Objective: Medical application of the new science of epigenetics, which explains how human genes are expressed and inherited as complex disease traits, holds high promise for individualized medicine. KLF11, a TGFbeta-

inducible tumor suppressor gene for hematological malignancies, which works via the Histone acetylase/deacetylase systems have been recently associated to leiomyomas. The following study seeks to define whether alterations in KLF pathways associate to the process of endometrial carcinogenesis.
Methods: Expression of KLF11 and its chromatin cofactors, was evaluated by immunofluorescence, immunohistochemistry, PCR and microarray-based profiling. Biochemical analysis of KLF-mediated pathways was investigated by targeted in vitro mutagenesis; mutant specificity was validated by GST protein-binding assays, promoter - reporter assays, chromatin immunoprecipitation and proliferation assays. All of the mechanistic studies involved the expression of KLF mutants in endometrial cancer cells. Genome-wide expression and epigenomic profiles were utilized to reveal selective molecular pathways that associated to the neoplastic phenotype in endometrial cells.
Results: Inactivation of the tumor suppressor KLF11 is found in human endometrial cancer. Functionally, disruption of KLF11 impaired regulation of cell growth. Bioinformatic analyses of genome wide profiles support a wider role for KLF11 in biological processes associated with carcinogenesis such as abnormal cell proliferation and dysregulation of apoptosis. Mutations which uncouple KLF11 from its epigenetic pathways revealed that many of its regulated processes can be manipulated both positively and negatively through novel pharmacological agents such as HDAC/HAT inhibitors which are being implemented in other cancer trials.
Conclusion: Patient data, cell biological studies as well as large scale genomic and epigenomic datasets reveal a role for KLF11 in endometrial cancer. These studies also provide valuable mechanistic information as to how KLF11 performs its functions. Lastly, the knowledge that these pathways are sensitive to novel pharmacological inhibitors is of therapeutic interest.

T-143

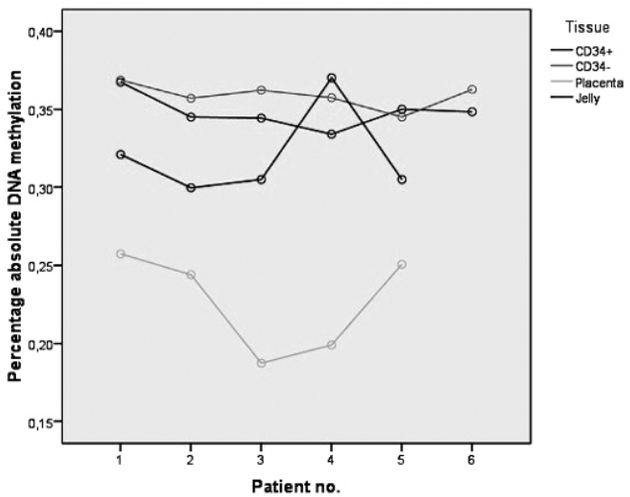
Effects of Gestational Protein Restriction on Expressions of DNA Methyltransferases in Rat Placental Labyrinth Zone. Haijun Gao, Uma Yallampalli, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
Gestational protein restriction has deleterious effects on placental development, resulting in long-term metabolic and cardiovascular consequences in offspring. DNA microarray with limited probe sets demonstrated that gestational protein restriction alters expression of genes in mouse placenta. These affected genes are related to cell growth and metabolism, apoptosis and epigenetic control, and more than half of them are down-regulated. It is known that DNA methylation in gene promoter region is one of the epigenetic mechanisms for gene expression. In this study, we hypothesized that gestational protein restriction alters the expression of DNA methyltransferases, key players in DNA methylation, in rat placenta. Pregnant Sprague Dawley rats were fed a normal diet (20% protein, control; n = 6) or a low protein diet (6% protein, PR; n = 6) from Day 3 of pregnancy until sacrificed at Day 18 of pregnancy. Labyrinth zone of placentas were dissected and snap-frozen for genes expression analysis by Real-time PCR. These genes include DNA (cytosine-5-)-methyltransferase 1 (*Dnmt1*), 3a (*Dnmt3a*, transcript variant 1 and 2) and 3b (*Dnmt3b*). The gender of placental tissues was determined by PCR on *sry* gene. The main findings include: 1) PR tended to increase the expression of *Dnmt1* in LZ ($P = 0.08$). *Dnmt1* mRNA levels in female LZ was 1.3-fold ($P < 0.05$) and 1.2-fold ($P < 0.05$) higher than those in male LZ in CT and PR rats, respectively; 2) The mRNA levels of *Dnmt3a* transcript variant 1 was increased by 1.3-fold ($P < 0.05$) by PR in female LZ by PR, but not in male LZ; 3) The mRNA levels of *Dnmt3a* transcript variant 2 were not changed by PR, however, in PR rats these mRNA levels were 1.3-fold ($P < 0.05$) higher in female LZ than male LZ; 4) The expression of *Dnmt3b* in rat LZ was not affected by diets and gender of placentas. These results indicate that expressions of DNA (cytosine-5-)-methyltransferases in rat LZ appears to be gender-specific, with higher expression of *Dnmt1* and *Dnmt3a* in female LZ and that PR may increase the methylation of genes in female LZ by Dnmt3a (Supported by National Institutes of Health grants R01HL102866 and R01HL58144).

T-144

Epigenetic Regulation of Molecular Pathways Relevant to Fertility: Accelerated Discovery of Disease Gene Networks and Therapeutic Targets. Adrienne Grzenda, Ravi Gada, Gaurang Daftary, Gwen Lomberk, Raul Urrutia. *ERDL (Epigenetic of Reproductive Diseases Labs), Department of Medicine, Ob/Gyn, Mayo Clinic.*
Objective: Epigenetics explains how human genes are expressed and inherited as complex disease traits and holds high promise for individualized medicine in fertility. We identified pathways critical for epigenetic reprogramming in organisms during gamete development and differentiation, namely KLF proteins and their cofactors Sin3/HDAC, HP1/SUV39/G9A, and EZH2/Polycomb. This study seeks to define epigenetic pathways that regulate fertility, individually and in cooperation, at the level of gene networks. Pharmacological agents targeting these molecules are used in clinical settings. As such, in assisted reproductive technology, these pathways may provide novel avenues for therapeutic intervention.
Methods: We developed maps of novel epigenetic pathways based on experimental high-throughput genomics and epigenomics datasets, specifically ChIP-on-Chip, ChIP-seq, and microarrays. Promoter occupancy and expression alterations were evaluated, providing high-resolution understanding of interaction between network nodes on a large scale. Multidimensional comparisons of genome, epigenome, interactome, and kinome were additionally performed to fully elucidate the nature of pathway connections. Pharmacological inhibitors and pathway-inactivating mutants to disrupt portions of the identified pathways were utilized. Our in vitro working pathway models were further supplemented and correlated with *in vivo* data generated in transgenic mice. Regulation of important pathway nodes was confirmed at the transcriptional and protein level by q-PCR and Western blot, respectively.
Results: We present the first high-resolution maps of new epigenetic pathways that participate in human reproduction. Mutational analyses and animal models confirm the validity of these maps, providing the most comprehensive understanding of these epigenetic regulators in mediating critical epigenetic events during gametogenesis from individual genes to complete networks. Pharmacological treatments demonstrate the ability to manipulate these pathways, affording exciting potential for future development in therapeutics.
Conclusion: Modeling of large scale genomic and epigenomic data, prototypes of which are presented, will significantly advance the field of reproductive science by accelerating the discovery of critical gene targets and networks disrupted in disease.

T-145

Tissue-Specific DNA Methylation Profiles in Newborns. E Herzog,[1] J Galvez,[1] A Roks,[2] L Stolk,[3] M Verbiest,[3] J Cornelissen,[4] E Steegers,[1] R Steegers-Theunissen,[1] Emilie Herzog. *[1]Obstetrics and Gynecology, Erasmus Medical Center (EMC), Rotterdam, Netherlands; [2]Farmacology, EMC; [3]Internal Medicine, EMC; [4]Hematology, EMC.*
Background: Intrauterine growth restriction increases the risk of chronic diseases in adulthood. Derangements in tissue-specific epigenetic programming of fetal and placental tissues are a suggested underlying mechanism, of which DNA methylation is best understood. DNA methylation profiles in human are mostly performed in DNA from white blood cells.
Objective: To assess DNA methylation profiles in placental tissue, umbilical cord blood stem cells and umbilical cord Wharton's jelly of human pregnancies.
Methods: We included 6 patients of which DNA was isolated from the fetal side of the placenta (n = 5), umbilical cord blood stem cells (CD34+ pluripotent stem cells, CD34- mononuclear cells (n = 6) and umbilical cord Wharton's jelly (n = 5). The blood stem cells were isolated using magnetic-activated cell separation. DNA methylation of CpG sites of the imprinted genes *IGF2 DMR/H19* and of the non-imprinted gene *VANGL-1* were performed in all tissues using a quantitative mass spectrometry method (Epityper, Sequenom). Statistical analysis using Kruskall Wallis test was performed.
Results: This data show statistically significant tissue-specific differences in DNA methylation in *IGF2 DMR* ($p<0.02$) and *H19* ($p<0.0001$) (figure: *H19* gene). This is mainly due to a higher methylation of *IGF2 DMR* in Wharton's jelly and a lower methylation of *H19* in placental tissue compared to other tissues. No statistical differences were observed in the very low methylated CpG sites of *VANGL-1*.



Conclusions: A significant tissue-specific variation of DNA methylation in 2 imprinted genes was demonstrated. Although these results have to be confirmed in larger sample sizes, the methods used point out the feasibility of tissue-specific epigenetic epidemiological studies in future. This will give the opportunity to investigate associations between pregnancy exposures and outcomes, chronic diseases in adulthood, and epigenetic profiles in human tissues.

T-146

HP1 Epigenetic Signals during Normal Spermatogenesis and Azoospermia. Phoebe H Leonard, Dean E Morbeck, Adrienne Grzenda, Ravi P Gada, Charles C Coddington, Gaurang S Daftary, Raul Urrutia, Gwen A Lomberk. *Epigenetics in Reproductive Diseases Labs (ERDL), Department of Ob/Gyn, Mayo Clinic.*

Objective: We seek to define how environmental signals mediate epigenetic reprogramming of gametes, critical for fertility. Heterochromatin Protein 1 (HP1) proteins are 'gatekeepers' of epigenetic signals through histone modifications (methyl-K9-histone H3 silencing). HP1γ is critical for primordial germ cell proliferation and spermatogenesis, and its alterations lead to azoospermia. Our studies have shown that HP1 is phosphorylated by Aurora A/PKA in response to environmental and cell autonomous signals which are epigenetically inherited. However, how HP1-mediated epigenetic signals are passed to progeny via sperm and their alterations decrease fertility remain to be determined.

Methods: Spermatocyte cells were utilized for RNA transcript and protein levels by qPCR and Western blot, respectively. Human and mouse testis were used for immunohistochemistry with P-Ser83-HP1γ and pan-HP1γ antibodies. Protein localization was observed in human, bull and mouse sperm by immunofluorescence and immunogold electron microscopy. Wild type HP1γ, S83A or S83D-carrying adenovirus was used to infect cells prior to RNA extraction for microarray analysis. The S83A mutant abolishes specific Aurora A/PKA-mediated phosphorylation of HP1γ, while S83D mimics a constitutively phosphorylated form of this protein.

Results: Spermatocytes express high levels of HP1γ and its phosphorylated Ser83 form. Examination in testis demonstrated that P-Ser83-HP1γ is more prominent in highly proliferative cells of this organ. Moreover, P-Ser83-HP1γ was localized to the midpiece of mature sperm in human, bull and mouse by immunofluorescence. Higher resolution imaging by electron microscopy revealed this phosphorylated subpopulation of HP1γ in the centriole and along the axoneme. Expression profiling performed using non-phosphorylatable (S83A) and phospho-mimetic (S83D) HP1γ mutants reveals that key gene expression networks regulate epigenetic reprogramming during spermatogenesis.

Conclusion: Our data provide evidence that appropriate transmission of HP1-mediated epigenetic signals is necessary for normal spermatogenesis. Ser83 phosphorylation of HP1γ plays a significant role in this process, and its disruption may, in part, participate in impaired spermatogenesis as seen in azoospermia. The concerted regulation of relevant gene networks further confirms its importance in fertility.

T-147

Epigenetic Modulation of microRNAs and Their Downstream Genes Expression in Leiomyoma and Leiomyoma Smooth Muscle Cells. Xiaoping Luo, Tsai-Der Chuang, Harekrushna Panda, Nasser Chegini. *OB/GYN, University of Florida, Gainesville, FL, USA.*

MicroRNAs are recognized as central players in various cellular processes. Aberrant miRNA expression is highlighted in many tumors, including leiomyomas. Studies found that histone modification associated gene silencing plays key roles in miRNA disregulated expression. Histone deacetylases (HDACs), including HDAC2 removes acetyl group from histone tail resulting in condensation of chromatin and suppression of gene transcription. To further understand the regulatory function of HDAC on miRNA in leiomyomas , we examined the expression of HDAC2, miR-18a, miR-20a, miR-26a and miR-133b in leiomyomas and matched myometrium (N=50) from proliferative and secretory phase of the menstrual cycle, from patients who received GnRHa, oral contraceptives (OCP) and progesterone therapies, and patients experiencing dysfunctional uterine bleeding. The results indicate that HDAC2 expression is elevated in leiomyomas from proliferative to secretory phase and in uterine bleeding patients as compared to myometrium. GnRHa, OCP and progesterone therapy down-regulate HDAC2 expression in myometrium and leiomyomas. The expression of miR-18a, miR-20a, miR-26a and miR-133b is inversely correlated with HDAC2 in above cohorts. HDAC2 inhibitors, TSA and SAHA, as well as HDAC2 siRNA significantly block the expression of HDAC2 and restore the expression of above miRNAs in leiomyoma smooth muscle cells (LSMC). Re-expression of these miRNAs inhibits cell proliferation by promoting Caspase 3/7 activity. Treatment of LSMC with TSA and SAHA suppresses the expression of CTGF, TGFßRII, SMAD4, and TGFß1, which are predicted/validated targets of miR-18a, miR-20a, miR-26a and miR-133b, respectively. HDAC2 inhibitors also reduce the expression of Transgelin2, MMP2, MMP9, which are downstream targets of TGFß signaling pathway. In conclusion, we demonstrate that miRNAs are epigenetically repressed in leiomyomas and altered as a result of hormonal therapies. HDAC inhibitors restore the expression of miRNAs, and therefore altered the expression of their downstream target genes which are essential for cell proliferation, differentiation and extracellular matrix turnover. Taken together, this study provides experimental evidence for epigenetic regulation of miRNAs and their target genes in leiomyomas and suggests HDAC as a potential therapeutic target in clinic Leiomyoma management (Supported by NIH grants HD37432, HD58664 and HD58779).

T-148

Evaluating the Incidence of NLRP7 Mutations in Hydatidiform Moles. Sangeetha Mahadevan,[1] Shu Wen,[2] Alfred Balasa,[3] Ignatia B Van den Veyver.[1,2,4] *[1]Interdepartmental Graduate Program in Translational Biology and Molecular Medicine, Baylor College of Medicine; [2]Departments of Obstetrics and Gynecology, ; [3]Pediatrics, ; [4]Molecular and Human Genetics, .*

Introduction: Hydatidiform moles (HM), a type of Gestational Trophoblastic Disease, are characterized by absent fetal development and a hyper-proliferative trophoblast with malignant potential. HMs are classified as Complete HM which are androgenetic diploid and Partial HM which are diandric triploid. While the incidence of most HMs are sporadic (~1/1,500 pregnancies) in the United States, a small subset of HMs are recurrent and biparentally inherited (BiHM). Autosomal recessive mutations of NLRP7 on chromosome 19q13.42 were identified as the pathological cause of BiHM. NLRP7 evolved from its ancestral gene NLRP2. Mutations in NLRP2 have been associated with trans-imprinting defects resulting in conditions like Beckwith Weidemann Syndrome. Others have suggested that variations in NLRP7 are the pathological basis of a variety of adverse reproductive outcomes. This has resulted in the need for further investigation of the consequence of NLRP7 mutations and how they contribute to reproductive success. The objective of this study is to characterize the mutational spectrum of NLRP7 in novel cases of Recurrent and Sporadic HM.

Hypothesis: Autosomal recessive, pathological mutations of NLRP7 are only or primarily associated with BiHM.

Materials and Methods: Sixteen affected women and eight unaffected spouses were recruited. Genomic DNA was isolated from peripheral blood/saliva. Eleven coding exons and exon-intron boundaries of NLRP7 were PCR amplified and subjected to Sanger Sequencing. The chromatograms were analyzed using Sequencher v4.7 sequence analysis software. Sequence variants were compared with those in the 1000 Genomes, dbSNP and HapMap databases. An Egyptian patient and one of African ancestry with BiHM were screened for an intragenic duplication previously described by our group. The functional effects of novel non-synonymous SNPs were evaluated using PolyPhen-2.

Results: Sequencing analysis of NLRP7 revealed previously described missense mutations, a novel homozygous loss of function mutation and a tandem intragenic duplication of exon 2-5 in the patients of Egyptian and African ancestry.
Future Directions: We plan to expand our study group to include other adverse reproductive outcomes and screen for mutations in NLRP7, NLRP2 and C6orf221, a gene recently implicated in BiHM.

T-149

Commonalities in Cardiac miRNA Expression in Baboon Fetuses Born to Over- and Under-Nourished Mothers. Alina Maloyan,[1] Jonathan AL Gelfond,[2] Mark J Nijland,[1] Peter W Nathanielsz,[1] Leslie Myatt. *[1]OB/GYN, University of Texas Health Science Center; [2]Epidemiology & Biostatistics, University of Texas Health Science Center, San Antonio, TX.*
Both maternal under- and overnutrition predispose offspring to cardiovascular disease (CVD) suggesting that these two paradigms share common pathological pathways. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression through translational repression. The involvement of miRNAs in CVD has been well documented. **We hypothesized that the similar cardiovascular phenotypic outcome of maternal under- and overnutrition would arise from commonalities in fetal cardiac miRNA expression.** Healthy female baboons of similar age and weight were randomly assigned to one of three diets: 1. Control diet throughout the pregnancy (C); 2. Maternal nutrient restriction (MNR) - 70% of control starting at 0.16 gestation (G); and 3. High fat/high fructose diet (HFD) starting 9 months prior to conception and throughout the pregnancy. All baboons underwent c-section near term (0.9G), and fetal hearts were collected. Fetal cardiac RNA from C (n=6), MNR (n=6) and HFD (n=5) groups was isolated, and miRNA sequencing and profiling performed.
We identified 70 differentially expressed miRNAs in HFD compared to C hearts ($p<0.05$), 50 upregulated and 20 downregulated. Compared to C, 53 miRNAs were differentially expressed in MNR hearts ($p<0.05$) with 39 upregulated and 14 downregulated. More pronounced differences in miRNA expression were found between MNR and C male (n=3), than female (n=3) fetuses, suggesting sexual dimorphism in the fetal response to MNR. Of all the differentially expressed and validated miRNAs that were homologous to those of humans, 7 were common between two paradigms, and have been previously linked to CVD. These included miR-143, miR-145, miR-378, miR-30, miR-133, miR-1-2, and miR-342. For all 7 miRNAs, 250 target genes were identified and input into Ingenuity Pathways Analysis to retrieve the target genes' association with CVD. Most of them were linked to coronary artery disease, cardiac hypertrophy, or heart failure (107, 54, and 33 genes respectively, $p<0.001$). Significant alterations occur in cardiac miRNA expression in the fetuses of both under- and over-nourished baboons with 7 of them being common suggesting that miRNAs may play a role in the fetal programming of CVD shared by both conditions of poor maternal nutrition. Supported by NIH PO1 HD21350, NIH P51 RR013986 and CTSA UL1RR025767.

T-150

Recurrent Pregnancy Loss in a Patient with Sex Chromosome Mosaicism: A Case Report and Review of the Literature. Vasiliki A Moragianni,[1,2] Michael M Alper.[1,2] *[1]Obstetrics & Gynecology, Division of Reproductive Endocrinology & Infertility, Beth Israel Deaconess Medical Center, Harvard Medical School; [2]Reproductive Endocrinology & Infertility, Boston IVF.*
OBJECTIVE
Only a few reports exist of an association between trisomy X, or Turner syndrome with subfertility and recurrent miscarriages. We report the case of a sex chromosome mosaicism associated with recurrent pregnancy loss in a patient seeking infertility treatment.
DESIGN
Case report.
MATERIALS AND METHODS
A 33-year-old G3P3003 female with a history of three early first trimester spontaneous miscarriages presented to our clinic for evaluation of subfertility. She reported regular menses and the last two pregnancies had been conceived after treatment with clomiphene citrate and timed intercourse. Her thrombophilia workup was negative, she had a normal hysterosalpingogram and pelvic ultrasound and her husband had a normal semen analysis.
RESULTS
Initial karyotype analysis of peripheral blood lymphocytes was consistent with 47,XXX/46,XX. After examination of an additional 100 cells, the karyotype was found to be 45,X/47,XXX/46,XX, comprising 2.5%, 3.3% and 94.2% consistently. Her husband's karyotype was 46,XY.
CONCLUSIONS
These findings emphasize the importance of karyotype analysis and genetic evaluation of any couple with recurrent miscarriages. This becomes especially important as offspring of sex chromosome mosaics are more prone to chromosomal aneuploidy.

T-151

Epigenetic Regulation of Histone Gene Expression – A Role in Hormone Therapy Resistant Breast Cancer? Shweta R Nayak,[1] Steffi Oesterreich,[1] Thushangi Pathiraja.[2] *[1]Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Magee Women's Research Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [2]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA.*
OBJECTIVE: To utilize a genome wide approach to characterize epigenetic changes associated with estrogen deprivation.
DESIGN: Basic Science Research
MATERIALS AND METHODS: Previously established MCF-7 cell clones, which are estrogen receptor (ER)-positive, were isolated after being cultured in estrogen-free media for 9 months (C4-12 cell line) and 18-24 months (LTED, Long term estrogen deprivation cell line); these daughter cell lines act as models for endocrine resistance, and while C4-12 cells are estrogen receptor (ER)-negative, ER is overexpressed in LTED cells. A genome-wide methylation screen was performed using Methyl CpG binding Domain (MBD) pull down assay followed by hybridization into Affymetrix Human Promoter 1.0R Array. Altered DNA methylation was validated by bisulfite genomic sequencing assays while gene expression of candidate genes was studied by qRT PCR and Western blotting.
RESULTS: 67 and 82 genes were hypomethylated in C4-12 and LTED respectively. In the C4-12 line, 4 of the 67 hypomethylated genes encoded for histone variants genes, while in the LTED line, 5 of the 82 hypomethylated genes encoded for histones variant genes. 1 histone variant, HIST1H2BE, a homomorphic variant of histone H2B, was found to be both hypomethylated in C412 and LTED cell line, and its overexpression confirmed with qRTPCR.
DISCUSSION: Estrogen deprivation in MCF-7 breast cancer cells results in altered promoter methylation. Significant changes include the hypomethylation, and subsequent overexpression, of genes which encode for histones variants. The altered expression of candidate histone target genes in breast cancer cells may cause changes in their associated chromatin. This change may lead to overexpression of various genes which may then enhance tumorigenesis or may lead to alternate pathways which may not only encourage cancer progression, but may also lead to cross talk between pathways, which would ultimately help the cell to survive in the absence of estrogen, and thus would contribute to acquired hormone therapy resistance in breast cancer patients.

T-152

Glucocorticoids Alter DNA Methylation in the Adult Male Germline: Implications for Fertility and Disease Programming in Offspring. Sophie Petropoulos,[1] Stephen G Matthews,[2] Moshe Szyf.[1] *[1]Pharmacology and Therapeutics, McGill University; [2]Physiology, University of Toronto.*
Background: The influence of environment and lifestyle on paternal germline is an emerging area of interest. Recently, molecular reprogramming of paternal germline was demonstrated in fathers who consumed a high-fat diet. In this study, female offspring developed diabetes-like disease, suggesting that paternal lifestyle modifies the sperm methylome. Synthetic glucocorticoids (sGCs) are commonly prescribed for the management of inflammatory and endocrine disorders, exposing thousands of males annually. However, nothing is known regarding the effects of GCs on germline methylome. We hypothesized that administration of sGC to adult males will alter global DNA methylation in mature sperm. It is likely that sGC via the glucocorticoid receptor target chromatin modifying enzymes to specific sites in the genome, precipitating changes in DNA methylation. Methods: Adult C57BL/6 males (n=5/group) were injected (s.c.) with dexamethasone (sGC; 1mg/kg) or vehicle (saline) for 5 consecutive days. Mice were split into two groups per treatment after the last injection and euthanized 35 days or 60 days later. Caudal epididymal sperm were collected and genomic DNA was extracted. LUminometric Methylation Assay (LUMA) was used to assess global DNA methylation. Trypan blue was used to determine cell death. Results: sGC administration resulted in a 1.9- and 1.6- fold (35 and 60 days, respectively) increase in sperm cell death versus control. LUMA revealed no significant difference in global DNA methylation 35 days post-treatment when compared to control. However,

at 60 days post-treatment, while no significant difference in HpaII/EcoRI digestion was observed (CpG methylation), there was a significant decrease in MspI/EcoRI digestion, suggesting a significant increase in CC methylation ($P<0.05$). Conclusion: We are the first to show that increased GC exposure can alter global DNA methylation of sperm in the adult male. We have also discovered that global modification of the sperm methylome following sGC exposure occurs in non-CpG sites. These findings are of crucial importance given that changes to the male germline methylome, may not only affect fertility and sperm function, but may also be transmitted to the embryo and program offspring for disease later in life. Further, such modifications may potentially be transmitted to subsequent generations.

T-153

An *ESR1* Mutation in a Female Is Associated with Delayed Puberty and Elevated Estradiol Levels. Samuel D Quaynor,[1] Earl W Stradtman II,[2] Jessie R Rubin,[1] Hyung-Goo Kim,[1] Sandra PT Tho,[1] Paul G McDonough,[1] Lynn P Chorich,[1] Derek A Schreihofer,[3] Lawrence C Layman.[1] *[1]Dept Ob/Gyn, Section Reproductive Endocrinology, Infertility, & Genetics; Institute of Molecular Medicine & Genetics, Georgia Health Sciences University, Augusta, GA, USA; [2]Pediatric & Adolescent Gynecology; Reproductive Endocrinology, Practice, Birmingham, AL, USA; [3]Dept Pharmacology and Neuroscience, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, USA.*
Disorders of complete androgen resistance (complete androgen insensitivity) have been described for many years in 46,XY individuals with mutations in the androgen receptor (*AR*) gene. However, the same is not true for estrogen resistance. Knockout of both the estrogen receptor-a (*Esr1*) and estrogen receptor-b (*Esr2*) genes has been reported in mouse, but there has only been one case of a mutation in the corresponding human *ESR1* ortholog in a male with complete estrogen resistance. No mutations have been identified to date in females, suggesting to some investigators that perhaps *ESR1* mutations are lethal in females. We have identified a 15 year old white female who presented with delayed puberty, absent (Tanner 1) breasts, Tanner 3 pubic hair, and normal appearing female external genitalia. On two occasions, serum estradiol was markedly elevated (3,500pg/mL and 756pg/mL); FSH = 6.7 and 14.5mIU/mL; LH = 9.6 and 9.1 mIU/mL. On ultrasound, her uterus was small, the endometrium was not identified, and the ovaries were enlarged and cystic. DNA was extracted from peripheral leukocytes and subjected to PCR-based DNA sequencing of the protein-coding exons. A homozygous missense mutation altering a completely conserved Cys residue was identified in the patient. This same nucleotide change was not seen in the SNP data base or controls. Preliminary *in vitro* experiments indicate that the mutant receptor binds estradiol normally, but that ERE-luciferase reporter expression is impaired compared with wild type. Our findings indicate that human *ESR1* mutations are compatible with life and result in a syndrome of estrogen resistance in females.

T-154

Day 5 Human Blastocysts Express a Unique and Sexually Dimorphic miRNA Profile. Evan M Rosenbluth,[1] Lane Christenson,[2] Eric J Devor,[1] Amy E Sparks,[1] Bradley J Van Voorhis.[1] *[1]Obstetrics and Gynecology, The University of Iowa; [2]Department of Molecular & Integrative Physiology, The University of Kansas.*
Objective: MicroRNAs (miRNAs) are small non-coding RNAs that are important post-transcriptional regulators and are known to be highly expressed in embryonic stem cells. MicroRNA sexual dimorphism has been demonstrated in stem cell lines but little is known about the role of miRNAs during human embryo development. The aim of this study was to identify the miRNA expression pattern in human blastocysts and to see if miRNAs were differentially expressed according to sex.
Design: Experimental
Materials & Methods: Cryopreserved pronuclear stage embryos previously donated with patient consent under an IRB approved protocol were thawed and cultured to day 5-6 blastocysts. Trophectoderm biopsies were performed and analyzed by array comparative genomic hybridization (CGH). The miRNA expression profile (754 miRNAs) of embryos was performed by quantitative real-time PCR with micro-fluidic Taqman®Low Density Array cards (Applied Biosystems). Differential expression was quantified using DataAssist™ software (v3 Applied Biosystems). Top differentially expressed miRNAs were confirmed by qRT-PCR utilizing single miRNA assays.
Results: A total of 11 female and 8 male blastocysts were analyzed. A total of 130 miRNAs were detected below our C_t value cutoff of 35. Many miRNAs of placental (C19MC cluster) and embryonic (miR302-367 cluster) origin were highly expressed in all embryos. There were 20 differentially expressed miRNAs ($p<0.05$) according to sex. The top differentially expressed miRNAs, including miR-512 and miR-518, were confirmed to show a 7-9 fold higher expression in male embryos ($p<0.05$).
Conclusion: Human euploid embryos express a unique miRNA profile and it appears that there is differential expression between male and female embryos as early as the blastocyst stage. In this investigation many of the highly expressed miRNAs are placental-specific and have been previously been detected in maternal serum during gestation. This study will serve as a starting point for future investigations that may identify potential gene targets and action of these miRNAs in early human development.

T-155

Differential Expression of micro-RNA in Day 5 Human Euploid and Aneuploid Blastocysts. Evan M Rosenbluth,[1] Lane Christenson,[2] Eric J Devor,[1] Amy E Sparks,[1] Bradley J Van Voorhis.[1] *[1]Obstetrics and Gynecology, The University of Iowa; [2]Department of Molecular & Integrative Physiology, The University of Kansas.*
Objective: micro-RNAs (miRNA) are small non-coding RNAs that are important post-transcriptional gene regulators and have been shown to be highly expressed in embryonic stem cells. Aberrant miRNA expression has also been observed in aneuploid cell lines. Since many chromosomally abnormal embryos fail to implant within the uterus, we hypothesize that miRNA may play a role in embryonic fate and the expression of miRNAs might be altered according to ploidy status. We aim to identify and confirm differential miRNA expression of human euploid and aneuploid embryos.
Design: Experimental
Materials & Methods: Cryopreserved pronuclear stage embryos donated with patient consent under an IRB approved protocol were thawed and cultured to day 5-6 blastocysts. Trophectoderm biopsies were performed on blastocyst-stage embryos and analyzed by array comparative genomic hybridization (CGH). Quantitative RT-PCR for 754 miRNAs/embryo was performed with micro-fluidic low density array cards and analyzed with DataAssist™ software (Applied Biosystems). Blastocysts developing from a second group of thawed/cultured embryos were biopsied and analyzed by CGH. Top differentially expressed miRNAs identified by the initial cohort were confirmed to be differentially expressed in the second embryo pool by single assay qRT-PCR.
Results: A total of 9 aneuploid and 19 euploid blastocysts were analyzed. Four biopsies produced chromosomally mosaic embryos and were excluded from the study. A total of 130 miRNAs were detected with C_t values less than 35. There were 22 differentially expressed miRNAs ($p<0.05$) according to ploidy status. The top miRNAs were confirmed to show a 4 to 27 fold higher expression in euploid embryos ($p<0.001$) by single qRT-PCR assays.
Conclusion: Depending upon chromosomal makeup, human day blastocysts express unique miRNA profiles. Interestingly, the most highly differentially expressed miRNA in euploid embryos (miR-141) is placental in origin and has been detected in maternal blood during pregnancy. This miRNA has also been shown to prevent cell death in cancer cells by blocking known apoptotic pathways. Downregulation in aneuploid embryos may facilitate apoptosis, ensuring non-viability prior to implantation. Further studies can help to elucidate the potential gene targets and roles these identified miRNAs play in early human development.

T-156

Epigenetics: New Insights into Postoperative Adhesion Development. Ghassan M Saed,[1] Nicole M Fletcher,[1] Douglas M Ruden,[1,2] Husam M Abu-Soud,[1] Michael P Diamond.[1] *[1]Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA; [2]Institute of Enviromental Health Sciences, Wayne State University School of Medicine, Detroit, MI, USA.*
Postoperative adhesion development involves a complex interaction of cytokines and growth factors. The genome contains primarily two forms of information: DNA sequence and epigenetic information, which includes histone and DNA methylation. We have previously characterized molecular differences between fibroblasts isolated from normal peritoneal and adhesion tissues of the same patient(s). The objective of this study was to determine epigenetic differences between these two cell lines.
Qiagen's EZ1 Advanced assay was used to isolate DNA from normal peritoneal and adhesion fibroblasts. Quantifiler Human DNA Quantification Kit (Applied Biosystems) was used to determine the amount of amplifiable DNA. Infinium Assay (Illumina) which measures DNA methylation levels at 27,578 CpG sites in over 14,000 genes was also used. A significant difference in methylation patterns was defined as at least 20% change in adhesion as compared to normal peritoneal fibroblasts.

Approximately 50% (7364 genes of 14,000 analyzed) have significantly different DNA methylation levels in adhesion as compared to normal peritoneal fibroblasts ($p<0.01$). 1287 genes with decreased DNA methylation had enriched GO categories, "Homeobox" and "Transcription Factor Activity". Of the 6077 genes with increased DNA methylation, 1685 genes had increased DNA methylation at least 50% in adhesion as compared to normal peritoneal fibroblasts and were enriched in the GO categories, "Glycoprotein" and "Defense Response."

Since 27,578 CpG sites are mostly in CpG islands and mRNA levels are inversely proportional to CpG island DNA methylation levels, DNA methylation changes likely cause global changes in gene expression levels. Demethylation of Hox and transcription factor genes in adhesion fibroblasts suggests that developmental programs of adhesions are dramatically altered compared to normal peritoneal fibroblasts. Methylation of "Glycoprotein" and "Defense Response" genes in adhesion fibroblasts suggests down-regulation of cell-surface proteins and immune defense systems compared to normal peritoneal fibroblasts. A greater understanding of the role of epigenetics in postoperative adhesion development will provide for improved interventions against this disease, which afflicts the majority of those who undergo abdominopelvic surgery.

T-157

Genome-Wide Methylation Profile Identifies Genes Involved in the Pathogenesis of Endometriosis. Hanyia Naqvi, Hugh Taylor. *Dept of OB/GYN, Reproductive Endocrinology, Yale University, New Haven, CT, USA.*

Objective: Endometriosis is associated with altered DNA methylation in the eutopic endometrium. Using a genome wide methylation screen, we identified methylated genes in the endometrium of women with endometriosis. Aberrant gene methylation may lead to alteration in the expression of genes contributing to the pathogenesis of endometriosis.

Methods: Endometrial biopsies were obtained from subjects undergoing treatment of endometriosis (n=6) or endometriosis-free controls (n=6). DNA was extracted from the eutopic endometrial tissue. 500ng of the genomic DNA were bisulphate converted using the Zymo Bisulphate conversion kit. Illumina Infinium HumanMethylation27, RevB Beadchip was used to survey the genome-wide methylation profile. 200ng of the converted DNA are amplified using the Illumina Infinium protocol and hybridized to the microarray for 16 hours. Hybridization was followed by a single base extension and fluorescent amplification using Tecan Freedom Evo. Chips were dried, scanned (Illumina iScan System) and analyzed. GenomeStudio was used for bioinformatics and statistical analysis.

Results: Of the 27,578 genes on the methylation array, 120 genes were significantly altered by a 1.5 fold change. 59 genes showed a statistically significance increase in methylation and 61 genes had a significant reduction in methylation ($p<0.05$). Genes up regulated include O-6-methylguanine-DNA methyltransferase (MGMT) and Dual specificity phosphatase 22 (DUSP22). Genes with decreased methylation include bone morphogenetic protein receptor (type IB) (BMPR1B) and tumor necrosis factor receptor 1B (TNFRSF1B). Changes in gene expression associated with altered methylation were validated using quantitative real time PCR (RTPcr).

Conclusion: Methylation has been demonstrated to play a role in the epigenetic regulation of gene expression. Global methylation profiling reveals a limited number of specific target genes in the endometrium of women with endometriosis. MGMT (negative regulator of ER), DUSP22 (regulates MAP kinase), BMPR1B and TNF receptor 1B are all aberrantly methylated in endometriosis; the altered expression of these genes may lead to abnormal regulation of the endometrial cell growth and function in women with endometriosis.

T-158

Altered Methylation in TNF Alpha Gene Associated with Early Normal Pregnancy but Not Preeclampsia at Delivery. Wendy M White,[1] Brian Brost,[1] Elizabeth Baldwin,[1] Jonathan O'Brien,[1] William Watson,[1] Carl Rose,[1] Norman Davies,[1] Zhifu Sun,[2] Stephen Turner,[3] Vesna Garovic.[3] *[1]Maternal Fetal Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA; [2]Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, Rochester, MN, USA; [3]Hypertension and Nephrology, Mayo Clinic College of Medicine, Rochester, MN, USA.*

BACKGROUND: Tumor necrosis factor alpha (TNFa) is an important proinflammatory mediator in the establishment and maintenance of normal pregnancy, as well as in preeclampsia. TNFa serum levels rise significantly in the first trimester of normal pregnancy and remain elevated over non pregnant states but decline with increasing gestation. Preeclampsia has been associated with increased levels compared with normotensive pregnant controls. Methylation is often inversely correlated with gene transcription. We sought to characterize methylation patterns in the TNFa gene and their association with normal and preeclamptic pregnancies.

STUDY DESIGN: The methylation profile of 2 CpG sites in the TNFa gene was characterized in DNA from maternal blood longitudinally in 14 women at <16 gestational weeks, at delivery and postpartum; in 14 nulligravid controls; and in 14 preeclamptics at delivery. All women were non-smokers and the three groups were matched for age and BMI. Genomic DNA was derived from buffy coat, purified, and bisulfite modified then run on the Illumina Methylation Assay. Mean methylation levels at each CpG site were compared using a t-test.

RESULTS: TNFa methylation pattern at both CpG sites did not differ significantly between the non-pregnant groups - postpartum vs nulliparous ($p=0.49$; 0.77). In contrast, TNFa CpG sites were significantly hypomethylated during early pregnancy as compared with the non-pregnant state ($p=0.0002$; 0.01). Methylation patterns in TNFa CpG sites did not differ significantly between normotensive and preeclamptic women at delivery ($p = 0.46$; 0.32).

CONCLUSION: Our results demonstrate that early pregnancy results in decreased methylation at two CpG sites, but methylation patterns return to baseline non-pregnant levels by the time of delivery. The presence of preeclampsia at the time of delivery does not alter methylation levels compared with normotensive pregnancies. This suggests that altered methylation may be an epigenetic mechanism to control TNF alpha production in normal early pregnancy, but not in preeclampsia.

T-159

Surveillance of Heterogeneity of Preterm Birth Rates in Central and Eastern Europe. Chander P Arora,[1,2] Marian Kacerovsky,[3] Ivana Musilova,[3] Levente Sara,[4] Balazs Zinner,[4] Decebal Hudita,[5] Iuliana Ceausu,[5] Kinga Lancz,[6] Ladislava Wsolovo,[6] Sukhveer S Sandhu,[1] Calvin J Hobel,[1,2] Vari G Sandor.[1] *[1]Ob-Gyn, Cedars-Sinai Med. Cntr., Los Angeles, CA, USA; [2]Ob-Gyn, David Geffen School of Med., UCLA, Los Angeles, CA, USA; [3]Ob-Gyn, University Hosp., Hradec Kralove, Czech Republic; [4]The 2nd Dept of Ob-Gyn, Semmelweis Univ., Budapest, Hungary; [5]Ob-Gyn, Dr. I. Cantacuzino" Hosp., "Carol Davila" Univ. of Med.& Pharmacy, Bucharest, Romania; [6]Environ. Med., Biostats. Anal., Res. Base of the Slovak Med. Univ., Bratislava, Slovakia (Slovak Republic).*

Background: The heterogeneity of term and preterm birth (PTB) in Central and Eastern Europe (CEE) is linked to the lack of surveillance of risk factors and inadequate data collection including the rates of mortality and morbidity. Partners of Mother and Child Health Research (M&CH) Network made an undertaking to identify the risk factors of preterm birth from four sites in CEE; Czech Republic, Hungary, Slovakia and Romania. One of the sites (Romania) is specialized high risk facility for PTB.

Objective: To identify and assess the risk factors leading to preterm birth in CEE European macro region.

Study Design: We extracted data from total of 26,012 term and 2724 preterm births (<37 wks) in a retrospective study, from 2007 to 2009 in four university hospitals of CEE. The study included historical risk factors, maternal complications and special tests during pregnancy.

Results: The data was analyzed using SAS to investigate Term and PTB frequencies for each core. Some of the parameters were not recorded in all the countries, due to the missing data in patient records. Mean PTB rate was 10.47% whereas the odds ratio for Czech was 3.3 times, for Hungary it was 3.1 times and for Romania it was 3.8 times higher than Slovakia. These estimates could give a likelihood ratio of the region.

After taking into account the differences in these four countries for preterm birth the logistic regression modeling shows the iron use, preeclampsia, history of smoking, and current diabetes are the significant predictors for preterm birth.

Conclusion: The collection of this important epidemiology data has already led to establishment of electronic data collection system (www.flexiform.hu). Data collection from four more sites in Croatia, Hungary and Ukraine is already in progress. The data would be pivotal to the public health authorities of the participating CEE countries to build robust PTB prevention initiatives.

T-160

The Andean Curse on the Conquistadors: A Comprehensive Study of High Altitude Hypoxia and Birth Weight in 24,827 Babies. R Soria,[1] CG Julian,[2] E Vargas,[1] LG Moore,[3] DA Giussani.[4] *[1]IBBA, La Paz, Bolivia; [2]University of Colorado, Denver, USA; [3]Wake Forest University, NC, USA; [4]University of Cambridge, UK.*

Investigations relating low weight or relative thinness at birth with increased rates of the metabolic syndrome in adult life have focussed on human populations undergoing maternal undernutrition (Yajnik. *Nutr Rev*. 2001;**59**:1) or famine (Roseboom et al. *Mol Cell Endo*. 2001;**185**:93) during pregnancy. Over 140 million people live at altitudes >3000 m, comprising the largest single human group at risk for fetal growth restriction due to hypoxia. However, no study has related high altitude-induced alterations in birth size to later cardio-metabolic diseases. We have created a cohort of 24,827 human birth records in Bolivia to serve as a platform to investigate such relationships. In this study, we determined in this cohort: 1) the effects of high altitude hypoxia on birth size and 2) whether high altitude native ancestry or female gender conferred any protection.

Methods: Records from deliveries between 1975-1985 were obtained over two years from obstetric clinics and hospitals in La Paz (3600 m) and Santa Cruz (420 m). Only singleton, healthy pregnancies of non-smoking mothers that reached term (>37 weeks) were assessed. Ancestry was determined by validated analysis of paternal and maternal surnames (Wilson et al. *AJP* 2007;**293**:R1313).

Results: High-altitude pregnancy reduced body weight and length at birth. High-altitude native ancestry conferred partial gradual protection on birth weight but not on length (Fig. 1). Consequently, babies born at high altitude of European ancestry were the thinnest for their length. The effects of high-altitude pregnancy on birth size were similar among male and female babies.

Conclusions: This comprehensive cohort of Bolivian birth records shows that high-altitude native ancestry but not female gender protects against the effects of high-altitude on birth size. Babies born at high altitude of European ancestry showed the most relative thinness at birth and, may therefore be the most susceptible to increased risk of disease in later life.

MRC and BHF, UK; NIH HL079647, USA.

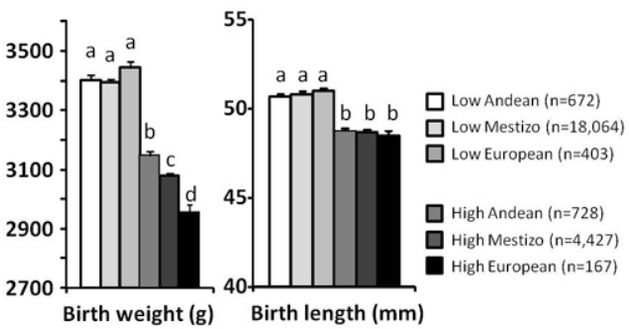


Fig.1. Mean+SEM for body weight and length at birth in 24,827 Bolivian babies. Different letters are significantly different. Newborn characteristics for male and female infants were compared between ancestry groups within each altitude as well as between altitudes within each ancestry group using GLM ANOVA in SPSS.

T-161

Evaluation of the Effects of Aqueous Herbal Extracts on the Viability of a Breast Cancer Cell Line. Fiona Smyth, Terry J Smith, Margaret O'Brien. *National Centre for Biomedical and Engineering Science, National University of Ireland, Galway, Ireland.*

Objective: Plant derived herbal medicines have been used for centuries. More than half of known anti-cancer drugs are derived from plants, e.g. the Madagscan periwinkle plant and the Pacific yew (1). Many herbal extracts are commercially prepared as alcoholic tinctures or experimentally extracted using complex methodologies. The availability and cost of preparation may preclude their use. Most herbal plants are traditionally used as teas. Our aim was to determine the effect of aqueous extracts of fresh herbs, which are readily available worldwide, on the viability of a breast cancer cell line, MCF-7.

Methods: Fresh material was harvested from feverfew, burnet, thyme, rosemary, sage, mint and hyssop plants and boiling water added for 15mins. MCF-7 cells were cultured in DMEM/FBS medium and experiments performed in phenol red-free DMEM and serum-stripped charcoal FBS for 24 and 48 hrs. Cell viability was determined using the MTT assay.

Results: Feverfew (33mg/ml) resulted in an 86% reduction in MCF-7 viability at 24hr (P<0.001) and 84% (P<0.001) at 48hr. After 24 hr treatment in feverfew at 17mg/ml and 3.3mg/ml concentrations, there were 78% (P<0.001) and 66% (P<0.001) reductions respectively, in viability. Burnet (33mg/ml) effected an 80% reduction (P<0.001) in cell viability which fell to 54% (P<0.001) at 17mg/ml, with thyme (17mg/ml-79%, P<0.001), rosemary (17mg/ml-68%, P<0.001), sage-(17mg/ml-57%, P<0.001), and mint (17mg/ml-41%, P<0.001), all at 24hr. Finally hyssop (33mg/ml) resulted in a 55% decrease at 24hr (P<0.001) and 72% (P<0.001) at 48hr.

Conclusion: All the herbs had significant cytotoxic effects on MCF-7 cells. Feverfew is traditionally used to treat fevers and headaches, parthenolide has been identified as one the main active ingredients, which aids in suppressing inflammation and is pro-apoptotic (2). An ethanolic feverfew extract was revealed to inhibit the growth of MCF-7 cells (3). Thyme, sage, rosemary and mint inhibited the growth of colon cancer cells (4). An ethanol extract of burnet has been demonstrated to have a strong anti-inflammatory effect involving the suppression of NFkB signalling events (5). Consequently, dietary herbs prepared in a cheap and simple way may have beneficial effects in terms of breast cancer treatment or perhaps in cancer prevention.

(1) Mans et al., 2000
(2) Mathema et al., 2011
(3) Wu et al., 2006
(4) Yi and Wetzstein, 2011.
(5) Yu et al., 2011

T-162

The Experience and Understanding of Illegal Abortion and Contraceptive Use amongst Poor Women in Argentina. Julia Zollner. *Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, United Kingdom.*

Introduction

Access to abortion services is restrictive, affecting significantly poor women, resulting in unsafe abortion being the number one cause of maternal mortality in Argentina, The relationship between contraception and illegal abortion is complex, influenced by sociocultural and gender role factors. It has been acknowledged that a woman's right to birth control includes access to safe abortion services and contraception, making it a public health priority.

Aims and Objectives

This qualitative study proposed to identify socio-cultural and gender-role factors affecting the complex relationship between contraception and illegal abortion of women of poor economic background in the city and province of Buenos Aires, Argentina, thus exploring their knowledge, attitude, and experience of a right to birth control.

Methods

9 semi-structured interviews were conducted, 5 in the city, and 3 in the province of Buenos Aires. Interviews covered participant's experience of contraception and abortion, and opinion on the legal situation of abortion in Argentina.

They were taped and transcribed verbatim to then explore narratives, according to proposed objectives, using thematic analysis.

Results

Male dominance was a major, and religion a minor determinant in the decision making process of abortion and contraception. A tight relationship between knowledge and effective use of contraceptive was found. The socio-cultural environment played an important role affecting women's opinion and acceptance of illegal abortion to regulate their fertility. There was lack of understanding of the current legal situation. Most women were against the legalisation of abortion, but accepted the de-penalisation in specific situations.

Conclusion

Contraception and illegal abortion are tightly linked to socio-cultural factors, access to contraceptives, and sexual health education and information. The illegal context will shape women's attitude, affecting their knowledge and understanding of the situation.

Proposed recommendations and further research in this study are warranted to provide better coverage and access to birth control in Argentina.

T-163

Transcription Factors FOXL2, GATA4, and Smad3 Co-Operatively Regulate Gene Expression Connected to Proliferation and Apoptosis in Ovarian Granulosa Cell Tumors. Mikko Anttonen,[1,2] Noora Andersson,[2] Marjut Pihlajoki,[2] Adrien Georges,[3] David L'hote,[3] Sanna Vattulainen,[2] Reiner A Veitia,[3] Markku Heikinheimo.[2] *[1]Dept. of Obstetrics & Gynecology, Univ. of Helsinki & Helsinki Univ. Central Hospital, Helsinki, Finland; [2]Children's Hospital, Univ. of Helsinki & Helsinki Univ. Central Hospital, Helsinki, Finland; [3]Institut Jacques Monod, CNRS-UMR 7592 & Université Paris-Diderot, Paris, France.*

Background: Granulosa cell tumors (GCT) represent 3-5% of ovarian cancer (incidence 4-10 per million) and bear a risk of late recurrence. The GCT pathogenesis started to get unraveled with the discovery of a missense mutation in FOXL2 (c.402C>G; C134W) in vast majority of GCTs. Wildtype (wt) FOXL2 inhibits proliferation and CyclinD2, and activates apoptosis in granulosa cells. In contrast, GATA4, previously connected to aggressive GCT behavior, inhibits apoptosis and activates Bcl2 in GCT cells. Both FOXL2 and GATA4 interact with Smad3, which is indispensable for CyclinD2 expression and granulosa cell proliferation.

Objective: We hypothesized FOXL2, GATA4, and Smad3 co-operate in the transcription factor complex involved in the (mis)regulation of proliferation and apoptosis in GCTs.

Design: Tissue microarray of 90 GCTs and mRNA of 29 GCTs were analyzed for FOXL2, GATA4, Smad3, CyclinD2, and Bcl2 expression. Physical and functional interactions were tested by immunopresipitation, and promoter transactivation assays in COS-7 and KGN (GCT derived) cell lines. The role of GATA4 and FOXL2 in proliferation and apoptosis of KGN cells was assessed using cell counting, BrdU, and caspase-3/7 activation assays.

Results: FOXL2, GATA4, and Smad3 expression positively correlate with each other, and with CyclinD2 and Bcl2 expression at mRNA and/or protein level; these factors are expressed at high/intermediate level in a majority of GCTs. Immunopresipitation verifies mutual physical interaction of FOXL2, GATA4, and Smad3. FOXL2 inhibits the synergistic CyclinD2 promoter transactivation by GATA4/Smad3 in COS-7 and KGN, whereas FOXL2 has an inhibitory effect on Bcl2 promoter transactivation by GATA4 only in COS-7 cells; wt and C134W FOXL2 do not have any differences in these respects. Finally, GATA4 significantly activates GCT cell proliferation and inhibits FOXL2 activated apoptosis.

Conclusions: FOXL2 and GATA4 exhibit opposite effects on proliferation and apoptosis regulation in GCT cells. The data indicates FOXL2, GATA4, and Smad3 are interconnected in the GCT pathogenesis.

T-164

Metformin Prevents Insulin Induced Cellular Proliferation and Activation of Signaling Pathways in Endometrial Epithelial Cells. Clare A Flannery,[1] Daryl J Selen,[2] Hugh S Taylor.[2] *[1]Endocrinology, Internal Medicine, Yale University School of Medicine, New Haven, CT, USA; [2]Obstetrics, Gynecology, & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Obesity and type 2 diabetes are independent risk factors for the development of endometrial hyperplasia and carcinoma. Hyperinsulinemia is a hallmark of obesity and type 2 diabetes, and may play a role in endometrial cell proliferation. In several case reports, metformin has reversed progestin-resistant endometrial hyperplasia. We sought to determine whether metformin has a direct effect on endometrial cells. We hypothesized that insulin activates signaling pathways, which initiate protein translation and cell proliferation, while metformin counteracts the effect of insulin through activation of AMPK.

Epithelial cells were separated from the endometrial tissues of ten women, and cultured in low glucose DMEM. Western blot analysis of phosphorylated and total AKT, MAPK, p70S6K, 4E-BP1, and AMPK was done after acute insulin stimulation in a dose range of 0.1 to 100nM for each group of epithelial cells. We then examined the relative concentrations of the same signaling proteins in cells pre-treated with 1 to 10mM of metformin, with and without insulin.

Primary epithelial cells demonstrated a four fold increase (p=0.02) in cell proliferation over 24 hours in the presence of insulin relative to vehicle. Treatment with metformin abolished the proliferative effect of insulin. Insulin induced a robust dose-dependent phosphorylation of AKT and p70S6K in all ten primary epithelial cell cultures. Insulin did not further increase the high baseline phosphorylation of MAPK or 4E-BP1. AKT & mTOR signaling was inhibited by wortmannin, a PI3 kinase inhibitor. Metformin treatment of epithelial cells reduced the insulin-induced phosphorylation of p70S6K, and activated AMPK. We have demonstrated that primary endometrial epithelial cells are sensitive to insulin, and that insulin likely promotes cell proliferation through activation of the AKT and mTOR pathways. Our data also provides preliminary evidence that metformin has a direct effect on the endometrium, potentially through activation of the AMPK pathway to inhibit insulin-induced cell proliferation and activation of signaling pathways. Metformin may be beneficial in the treatment of endometrial hyperplasia for women with hyperinsulinemia, in the setting of obesity and type 2 diabetes.

T-165

Adolescent Understanding and Acceptance of the HPV Vaccination in an Underserved Population in New York City. Jill Blumenthal,[1] Melissa K Frey,[2] Michael J Worley,[3] Nana E Tchabo,[4] Karen Soren,[5] Brian M Slomovitz.[4] *[1]Internal Medicine, New York Presbyterian Hospital Weill Cornell Medical College, New York, NY, USA; [2]Obstetrics and Gynecology, New York Presbyterian Hospital Weill Cornell Medical College, New York, NY, USA; [3]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA; [4]Brigham and Women's Hospital, Morristown Memorial Hospita, Morristown, NJ, USA; [5]Pediatrics, New York Presbyterian Hospital Columbia, New York, NY, USA.*

Objective: HPV vaccination may prevent thousands of cases of cervical cancer. We aimed to evaluate the understanding and acceptance of the HPV vaccine among adolescents.

Methods: A questionnaire was distributed to adolescents at health clinics affiliated with a large urban hospital system to determine knowledge pertaining to sexually transmitted diseases and acceptance of the HPV vaccine.Results: 223 adolescents completed surveys. 28% were male and 70% were female. The mean age for respondents was 16 years old. Adolescents who had received the HPV vaccine were more likely to be female and to have heard of cervical cancer and Pap testing. Of the 143 adolescents who had not yet been vaccinated, only 4% believed they were at risk of HPV infection and 52% were willing to be vaccinated.

HPV Vaccine Status

	Have had HPV vaccine (n, %)	Have not had HPV vaccine (n, %)	Dont' know (n, %)	P Values
Gender				<0.001
Male	2 (3.1)	60 (93.8)	2 (3.1)	
Female	71 (45.2)	84 (53.5)	2 (1.3)	
Heard of Cervical Cancer				<0.001
Yes	69 (41.1)	97 (57.7)	2 (1.2)	
No	4 (7.5)	47 (75.2)	1 (0.1)	
Heard of Pap Screening				0.003
Yes	44 (42.7)	56 (54.4)	3 (2.9)	
No	28 (23.9)	88 (75.2)	1 (0.1)	
Understand the Goal of Pap Screening				0.003
Yes	29 (50.9)	27 (47.4)	1 (1.8)	
No	43 (26.7)	115 (71.4)	3 (1.9)	
Heard of Sexually Transmitted Disease				0.241
Yes	69 (34.2)	130 (64.4)	3 (1.5)	
No	4 (21.1)	14 (73.7)	1 (5.3)	
Have had Other Vaccines				<0.001
Yes	35 (72.9)	12 (25.0)	1 (2.1)	
No	33 (22.6)	112 (76.7)	1 (0.7)	
Unsure	2 (16.7)	8 (66.7)	2 (16.7)	
Believe at Risk for Abnormal Pap / Cervical Cancer				0.333
Yes	8 (57.1)	6 (42.9)	0 (0)	
No	43 (31.2)	92 (66.7)	3 (2.2)	
Unsure	16 (28.6)	39 (69.6)	1 (1.8)	

Conclusions: Surveyed adolescents demonstrated a marginal willingness to receive the HPV vaccine and a lack of awareness of personal risk for acquiring HPV.

T-166

Understanding and Acceptance of the Human Papilloma Virus (HPV) Vaccine among HIV-Infected Adolescents. Jill Blumenthal,[1] Melissa K Frey,[2] Constance Rubow,[3] Sima S Toussi.[3] *[1]Internal Medicine, New York Presbyterian Hospital Weill Cornell Medical College, New York, NY, USA; [2]Obstetrics and Gynecology, New York Presbyterian Hospital Weill Cornell Medical College, New York, NY, USA; [3]Pediatric Infectious Disease, New York Presbyterian Hospital Weill Cornell Medical College, New York, NY, USA.*

Objective: HIV-infected women have been shown to be at increased risk for cervical HPV infection and cervical dysplasia. The HPV vaccine is available for females and males age 9 through 26 in the United State. Population based studies have shown that over 60% of teenagers are sexually active by grade 12. The purpose of this study was to evaluate the understanding and potential acceptance of the HPV vaccine by HIV-infected individuals aged 13-24 years old to determine the level of understanding of HPV infection and the vaccine.

Methods: From 2009-2010, HIV-infected patients were recruited from a pediatric HIV health clinic affiliated with a large urban hospital to participate in a questionnaire that assessed knowledge pertaining to vaccinations, sexually transmitted diseases and cervical cancer. Descriptive statistics were compared using the chi-square and fisher exact tests.
Results: 36 of 50 HIV-infected patients completed the survey. 53% (19) were female. 58% (21) were African American. 75% (27) had heard of cervical cancer and 64% (23) had heard of Pap smears. 81% (29) where aware of the mechanism of HPV transmission. 33% (12) reported having received the HPV vaccine. Of the 17 patients who had not been vaccinated, 71% (12) were willing to get vaccinated, 24% (4) were unsure, and only 1 patient was unwilling to be vaccinated. 95% of the females were willing to get vaccinated while 59% of the males were willing to get vaccinated. Only 28% (10) of participants felt that they were currently at risk for acquiring HPV.
Conclusions: This study demonstrated a strong willingness of HIV-infected adolescents to receive the HPV vaccine. Despite being at increased risk of acquiring HPV, there was a lack of awareness of personal risk for acquiring HPV. High-risk adolescents' understanding of cervical cancer and HPV has improved, but gaps remain. Improved knowledge of HIV-infected adolescents' and young adults' understanding and acceptance of the HPV vaccine would help us provide appropriate and useful information to parents, providers and young patients when making the decision to vaccinate.

T-167

Routes of HPV Dissemination in Patients with Cervical Intraepithelial Neoplasia (CIN). Evgeniy A Kogan, Nafisa M Faizullina, Tatiana A Demura, Larisa S Ezhova. *Anatomic Pathology, Research Center of Obstetrics Gynecology and Perinatology.*
The routes of HPV dissemination in patients are of great importance. Literature data show presence of viral DNA also in endometrial samples and in blood. (P.Kay et all, 2005). Background: The objective of the study was to investigate possible routes of HPV dissemination in patients with CIN.
Materials and Methods: Surgical and biopsy material was used from 33 patients with CIN associated with HPV infection. Morphological evaluation of paraffin sections was performed with following immunohistochemical (IHC) analyses using antibodies to P16 and Ki67. HPV DNA was assessed by PCR in situ. Primers were designed to amplify the E6 region of HPV16. Biotin-labeled dUTPs were incorporated during PCR amplification, and subsequently amplicons were detected with peroxidase-streptavidin conjugate.
Results: Morphological and IHC analyses revealed CIN of different grades: CIN I in 10 cases, CIN II in 10 cases and CIN III in 13 cases. In all cases underlying connective tissue was highly infiltrated by lymphocytes, macrophages and contained numerous vessels of capillary type (increased angiogenesis). bPCR in situ showed HPV DNA localization not only in epithelial cells, but also in macrophages and endothelial cells.
Conclusion: Obtained results give us understanding of viral dissemination in patients, that is based on HPV contamination of cells able to circulate in blood and lymph. These data show possible haematogenic, lymphogenic and transplacental spread of HPV in infected patients. That type of infection process generalization may lead to HPV associated tumors in different organs and infection in newborns.

T-168

Origin of Leiomyocytes and Adipocytes in Lipoleiomyoma. Evgeniya A Kogan, Nafisa M Faizullina, Tatiana A Demura, Nataliya V Nizyaeva, Vladimir B Nosov, Anna V Babkina. *Anatomic Pathology, Research Center of Obstetrics Gynecology and Perinatology.*
Lipoleiomyomas (LLMs) are known to be rare uterine neoplasms composed of smooth muscle and adipocytes. The origin of this tumor is still controversial. Majority of pathologists consider that fat tissue in LLM result from progressive intracellular accumulation of lipids in smooth muscle cells, while others speculate that the origin of this tumor cells is connected with immature mesenchimal cells. The aim of our study was to investigate the immunohistochemical phenotype of uterine LLMs and to determine origin of tumor leiomyocytes and adipocytes . Tissue samples of 12 uterine lipoleiomyomas were taken from patients ranged 29-51 yy. (mean age 37). We have studied tumors on gross inspection, histologically and immunohistochemically for vimentin, desmin, SMA, CD34, OCT-4, PTEN, Ki-67. All tumors were composed from irregular bundles of smooth muscle cells and mature large adipocytes. Adipose component varied from 5 to 75% of the tumor mass. Adipocytes localized both in groups and single cells in perivasculare region. Smooth muscle cells of tumor were highly positive for desmin, SMA, PTEN. However Ki-67 was revealed in single cells. Adipocytes in the tumors demonstrated vimentin expression. We also found immunoreactivity of CD 34, OCT4, Ki-67 in single cells in adjacent tissue to adipocytes. LLMs didn't show expression of desmin, PTEN and SMA. Conclusion: Obtained results show that adipocytes of LLM may originate from mesenchymal stem cells. Perivascular localization of tumor adipocytes rises the question: weather this immature mesenchymal cells have histiogenic or haematogenic origin.

T-169

Estradiol-17β and Its Metabolites Attenuate L-Ascorbic Acid-Suppressed Human Ovarian Cancer Cell Growth. Huihui Li, Yingjie Zhao, Yan Li, Caifeng Dai, Sheikh O Jobe, Ronald R Magness, Jing Zheng. *Dept. of Ob/Gyn, Univ. of Wisconsin, Madison, WI, USA.*
Estradiol-17β ($E_2β$) plays a critical role in the growth, invasion and metastasis of human ovarian cancer cells. However, it is unknown whether its biologically active metabolites 2-hydroxyestradiol (2-OHE_2), 4-hydroxyestradiol (4-OHE_2), 2-methoxyestradiol (2-ME_2) and 4-methoxyestradiol (4-ME_2) have similar actions on human ovarian cancer cells. It has also been shown that L-ascorbic acid (AA) significantly inhibits ovarian cancer cell growth *in vitro*, but the reports on AA's *in vivo* effects on ovarian cancer are unclear. Herein, we examined expression of cytochrome P450s and catechol-*O*-methyltransferase (COMT) as well as if $E_2β$ and its metabolites and AA interactively modulate cell proliferation in OVCAR-3, SKOV-3, OVCAR429, OVCAR432 and IOSE385 cells. ***Methods:*** Western blotting was used to confirm expression of CYP1A1, CYP1B1, COMT, ERα and ERβ in these cells. Cells were treated with $E_2β$ and its metabolites (0.01-100 nM) for 6 days or AA (65-2000 μM) for 4 days. Cell proliferation was evaluated using crystal violet. Interactive effects of $E_2β$ and its metabolites with AA were also evaluated. ***Results:*** **1)** CYP1A1, CYP1B1, COMT, ERα and ERβ were expressed in all four ovarian cancer cell lines studied; **2)** $E_2β$ and its metabolites stimulated ($P \leq .05$) cell proliferation (~25%) in OVCAR-3 and IOSE383, but not SKOV-3, OVCAR429 and OVCAR432 cells with the maximum stimulatory effect at 0.1 nM; **3)** AA dose-dependently suppressed ($P \leq .05$) cell proliferation in OVCAR-3, SKOV-3, OVCAR432, and IOSE385. In OVCAR429 cells, AA enhanced cell proliferation at doses < 125 μM, whereas attenuated cell proliferation at doses ≥ 125 μM; **4)** $E_2β$, 4-OHE_2 and 4-ME_2, but not 2-OHE_2 and 2-ME_2 partly attenuated ($P \leq .05$) the AA's suppressive effect on growth of OVCAR-3, SKOV-3, OVCAR432 and IOSE385, but not OVCAR429 cells. ***Conclusions:*** $E_2β$ and its metabolites promote cell proliferation only in one ovarian cancer cell line studied, whereas AA inhibits cell proliferation in all four ovarian cancer cell lines studied. Moreover, $E_2β$, 4-OHE_2 and 4-ME_2 attenuate AA-suppressed cell proliferation in three cancer cell lines studied. These data indicate that $E_2β$ and its metabolites stimulate growth of some ovarian cancer cells; however, $E_2β$ and its metabolites may play a more important role in rescuing ovarian cancer cells when challenged with AA or other anti-cancer agents, which will necessitate more research.

T-170

Fertility-Sparing Treatment of Endometrial Cancer Precursors among Young Women: A Reproductive Point of View. Enzo Ricciardi,[1] Paolo Maniglio,[1] Filippo Bellati.[2] *[1]Salute della Donna e Medicina Territoriale, Sapienza Universita' Di Roma, Italy; [2]Scienze Ginecologico-Ostetriche e Scienze Urologiche, Sapienza Universita' Di Roma, Italy.*
OBJECTIVE: To assess, in women below the age of 40 with diagnosis of atypical endometrial hyperplasia, conceiving rate, oncologic risk of delaying definitive treatment and pregnancy related complications.
DESIGN: Prospective clinical study
SETTING: Tertiary University Hospital.
PATIENT(S): 15 women below the age of 40 with complex atipica hyperplasia or early stage endometrial carcinoma with desire to preserve fertility.
INTERVENTION(S): Progestins administered orally for at least a 12-weeks period.
MAIN OUTCOME MEASURE(S): Endometrial biopsies at follow-up.
RESULT(S): In 11 women, a complete pathological remission of the disease was observed. 4 pregnancies were attained in 4 women. 3 showed progression and underwent definitive surgery at 18 months. 1 showed no response at 24 months and 3 cycles and was ardessed to hysterectomy.
CONCLUSION(S): A conservative approach in patients below the age of 40 appears feasible and safe. This approach requires a strict monitoring in order to avoid possible detrimental effects on servival.

T-171

COUP-TF II and Beta-Catenin in Human Uterine Fibroids. Marina Zaitseva,[1] Sarah J Holdsworth-Carson,[1] Julia Rubulis,[1] Beverley J Vollenhoven,[2] Peter AW Rogers.[1] *[1]Gynaecology Research Centre, Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia.*

Background. Uterine fibroids (leiomyoma) are benign neoplasms of myometrium. Their etiology is poorly understood, but they are known to be sex steroid hormone dependant. Our previous studies have reported that COUP-TFII and β-Catenin are altered in fibroids compared to myometrium. Both COUP-TF II and β-Catenin have established roles in tumorigenesis and pathophysiologies of the reproductive tract. Furthermore, it has been demonstrated that constitutive over-expression of β-Catenin leads to mesenchymal tumors in mice, and that β-Catenin, retinoic acid (RA) and Sonic Hedgehog (Shh) regulate COUPTF-II expression.

Objective: To investigate COUPTF-II and β-Catenin expression *in vivo* and regulation *in vitro* in human myometrium and fibroids.

Methods: Samples were collected from pre-menopausal, cycling women with no hormone therapy for 3 months prior to surgery. COUP-TF II and β-Catenin mRNA expression were analysed by qRT-PCR and protein expression and localisation by Western blot and immunohistochemistry (IHC). For regulation studies, myometrial and fibroid primary cultures were stimulated by Shh, P (100nM) and/or RA (1μM) for 24 h. Gene expression was analysed by RT-qPCR.

Results: COUPTF-II mRNA expression was reduced in fibroids compared to myometrium, while protein levels were unchanged. Both β-Catenin mRNA and protein were higher in fibroids compared to myometrium. Semi-quantitative IHC scoring demonstrated significantly stronger expression in myometrial vasculature compared to the rest of the tissue for both β-Catenin and COUPTF-II, while in fibroids only COUPTF-II was more strongly expressed in the vessels. In vitro studies demonstrated that while the Shh pathway was activated, it did not affect COUPTF-II or β-Catenin expression in myometrial or fibroid cells, while P and RA significantly decreased COUPTF-II but not bCatenin expression in myometrial but not in fibroid cells.

Conclusion: The data presented supports a possible role for COUP-TF II and β-Catenin in the development of uterine fibroids. Aberrant expression of transcription factors, COUP-TF II and β-Catenin can cause dysregulation of target gene expression, leading to prolific fibroid growth. However, further work is required to better map the relationship between these factors and uterine fibroid pathophysiology.

T-172

Development of Viral Phage Technology for Targeted Imaging and Therapy Against Gynecologic Malignancies. Jacob Rotmensch, Liaohai Chen. *Section of Gynecologic Oncology, Rush University Medical Center, Chicago, IL, USA.*

Viral phages are investigated for delivery of targeted therapy against gynecologic malignancies. T7 viruses are fast growing and extremely stable double strand DNA lytic phages that can be used as carriers for imaging and radionuclide therapy. In this study, hybrid isotope-containing phage particles have been engineered that have a high affinity binding to a specific molecular targets and may have potential to image or treat gynecologic malignancies in a novel way.

T7 ghost viral phages as the template for the synthesis of radiophage have been fabricated. T7 ghosts were made by osmoticly shocking normal T7 phages. Capillary zone electrophoresis confirmed the generation of T7 ghost phages. Based on the peak areas of the ghost particles and intact phages, the yield of ghost phages was approximately 55%. To visualize these ghost particles, phage samples prepared were negatively stained with uranyl acetate (1%) and examined by Transmission Electron Microscopy (TEM).

To establish radiophages capable of delivering targeted radionuclide agents for therapy, the T7 ghost phages described above were used as templates to grow metallic cobalt inside the core to occupy the volume that was taken up by DNA in intact phages. The incorporation of cobalt into the empty core of the phages was achieved by reducing cobalt ions (II) with sodium borohydride. To visualize the cobalt containing hybrid viruses, samples were immobilized on a gold-coated grid and imaged by TEM and by energy dispersive x-ray (EDX) analysis.

Also, ^{99m}technetium oxide hybrid radiophages were engineered that may be useful for targeted cellular imaging. The synthesis of ^{99m}technetium oxide particles was achieved by reducing potassium pertechenate (VII) with sodium borohydride. The formation of these hybrid phages were confirmed by TEM imaging which showed uniform ^{99m}technetium oxide particles (~40nm) inside the ghost viral T7 shells.

In another experiment, in order to maximize sensitivity for molecular MRI imaging, superparamagnetic nano-particles of iron (Fe) were incorporated in the T7 ghost viruses. This allowed for formation of viral particles that can be detected in a magnetic field. These superparamagnetic hybrid phages were then placed in such a magnetic field and detected with great sensitivity.

These studies show that viral phage technology can be used as a novel way to deliver targeted imaging and therapy against gynecologic malignancies.

T-173

Partnership To End Disparities in Minority Biospecimen/Biobanking: The Region 5 NCI BMaP Story. Melissa A Simon,[1] Linda Fleisher,[2] Erika E De La Riva,[1] Carrie Norbeck,[2] DelRoy Louden,[3] J Robert Beck,[2] Raymond Bergan,[1] Julian C Schink,[1] Region 5 G/BMaP Partners. *[1]Northwestern Univ Feinberg School of Medicine-Robert H. Lurie Comprehensive Cancer Center; [2]Fox Chase Cancer Center; [3]Lincoln University.*

Background: The Region Five GMaP/BMaP Network is a partnership representing 18 NCI funded institutions committed to planning a state-of-the-art network dedicated to ensuring adequate and continuous supply of high-quality human biospecimens from multi-ethnic communities. This Network brings together community-engaged researchers, basic scientists, biospecimen experts, bioinformatics researchers, community members and organizations to address challenges in biospecimen research.

Methods: Leveraging a community-engaged approach, the Comprehensive Needs Assessment (CNAT) was developed to assess minority biospecimen collection, biobanking practices, education, and outreach initiatives. CNAT consisted of a mixed methods approach utilizing 4 survey and interview instruments targeting PI's and biospecimen facility administrators Mar-Oct 2011.

Results: The survey was completed by 10 Region Five biospecimen facility administrators. Preliminary findings consistently reported disparities in biospecimen collection among minority populations. Gaps in specimens collected for populations with a high burden of cancer were identified. Seven facilities reported collecting specimens from 116,417 White vs. 12,592 Non-White patients- 1648 were Hispanic. Forty percent of the biospecimen facilities have collaborated on biospecimen education efforts. Biospecimen facility administrators identified several barriers which limit them from collaborating in collection programs including the wide range of data systems/ platforms that are not necessarily compatible with each other. However, they also indicated a high desire to collaborate.

Conclusions: There is a need to focus on minority biospecimen collection efforts and address the gaps in how race and ethnicity and subtypes of biospecimens are collected across our institutions. Entering the 3rd year with NCI funding, Region Five BMaP will continue to focus on strengthening collaborative relationships with biospecimen facilities and other stakeholders while fostering further biospecimen research focused on eliminating cancer disparities.

Funding Acknowledgement: 5U01 CA116875-05S4

T-174

The Functional Impact of Progesterone Receptor Alternative Splicing in Breast Cancer Cells. David MW Cork,[1] Thomas WJ Lennard,[2] Alison J Tyson-Capper.[1] *[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; [2]Northern Institute of Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Introduction: The progesterone receptor (PR) gene consists of eight exons encoding the PR-A and B isoforms which function as classical ligand activated nuclear transcription factors with differing activities. PR-B may additionally function non-genomically via cross-talk with cytoplasmic signalling molecules. Progesterone, via PR, is an important mitogenic signal during mammary gland development and the balance of PR isoform expression is vital for maintaining normal development. Disruption of the PR isoform ratio is frequently observed in the early stages of breast cancer, implicating PR signalling in disease development. The PR status of breast tumours, measured by immunohistochemistry, is a biomarker for efficacy of endocrine therapies and may be indicative of disease prognosis. Alternative pre-mRNA splicing involving deletion of internal exons and intron retention has been reported for PR, potentially generating functionally distinct truncated proteins.

Methods: PR mRNA expression was assessed in the breast cancer cell lines MCF-7 and MDA-MB-231, and in breast tumour tissue, by RT-PCR. PR protein

expression was characterised by immunoblotting and immunofluorescent staining. Potential functionality of PR proteins in MDA-MB-231 cells was assessed by ligand blotting, co-immunoprecipitation and DNA affinity precipitation assays.
Results: We report the presence of a range of PR mRNA in MDA-MB-231 cells, showing that PR undergoes extensive alternative splicing in this reportedly PR negative cell line. These transcripts may encode the low molecular weight nuclear and cytoplasmic PR proteins detected in these cells. We further show that these proteins are capable of binding progesterone, interacting with the PR nuclear co-factor PSF, dimerising and binding DNA. These truncated PR isoforms may therefore function via classical or non-genomic pathways but function differently to full length PR. Notably, we also identify novel and previously described alternatively spliced PR mRNA in breast tumours, including samples originally characterised by immunohistochemistry as being PR negative.
Conclusions: Alternative splicing generates functional truncated PR isoforms which may alter the progestin response of cells/tissues and potentially affect the detection of PR for use as a breast cancer biomarker.

T-175

Identification of SAFB1 and SAFB2 Target Genes within the Oestrogen Receptor-Signalling Pathway in Breast Cancer Cells. Elaine A Hong,[1] David J Elliott,[2] Alison J Tyson-Capper.[1] [1]*Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom;* [2]*Institute of Genetic Medicine, International Centre for Life, Newcastle University, Newcastle upon Tyne, United Kingdom.*
Background: The scaffold attachment factors SAFB1 and SAFB2 are multifunctional DNA- and RNA-binding proteins involved in a wide range of biological processes such as cell cycle regulation, apoptosis, differentiation, stress response and also RNA processing. Both SAFB1 and SAFB2 are linked with breast cancer through genetic analysis and their function as oestrogen receptor (ER) co-repressors. The main objectives of this study were to identify gene targets for these two proteins in invasive breast cancer cells. To address these aims we used a combined approach of immunoblotting, siRNA mediated gene silencing and Q-PCR arrays using non-invasive MCF-7 and invasive MDA-MB-231 breast cancer cells.
Results: Our data from immunoblotting experiments show that both SAFB1 and SAFB2 expression increases in response to beta-oestradiol treatment ER positive MCF-7 breast cancer cells and decreases in MDA-MB-231 breast cancer cells (reportedly to be ER negative). To identify potential gene targets for these proteins we first depleted MDA-MB-231 cells of SAFB1 or SAFB2 by RNAi and then utilised an ER-signalling focused pathway Q-PCR array system. The array experiments were performed in triplicate and data analysed for fold changes in gene expression using the ΔΔCt method. Up-regulated targets were defined as those with a fold change > 2.0 and down regulated targets with a fold change < 0.5. Validation of the array data was undertaken by Q-PCR, using Taqman probes, for individual genes. These experiments identified novel transcriptional hits for both SAFB1 and SAFB2; most of the target genes including CLU, ESR1, IL2RA, KIT, KLK5 and SERPINB5 were up-regulated in the absence of SAFB1, SAFB2 or both but only IL6 was down-regulated. These target genes have major roles in apoptosis, hormone receptor signalling and regulation of the immune system.
Conclusions: Characterisation of these new target genes supports previously reported evidence that indicates the primary role of SAFB1 and SAFB2 as transcriptional co-repressors and has identified potential molecular targets which correspond with a possible active role of these proteins in the progression of breast cancer.

T-176

Novel Insights into the Genetics of Early Spontaneous Preterm Birth Using Multigenic Modelling. Qi W Ang,[1] Melanie K Slater,[1] Ramkumar Menon,[3] Stephen J Lye,[2] Mario Merialdi,[5] Craig E Pennell.[1] [1]*School of Women's and Infants' Health, The University of Western Austraia;* [2]*Samuel Lunenfeld Research Institute, University of Toronto;* [3]*Department of Ob & Gyn, The University of Texas Medical Branch;* [4]*Reproductive Health, World Health Organization.*
Objective: Understanding the role of genetics in spontaneous preterm birth (PTB) has been difficult as most studies to date utilized a candidate gene approach with limited replication of their results. The objective of this study is to identify maternal genetic variants associated with *early* PTB 24–34 weeks gestation, utilizing candidate genes from the PTBGene electronic database (http://bioinformatics.aecom.yu.edu/ptbgene/index.html) in 1174 samples from the Preterm birth Genome Project (PGP).
Methods: Maternal DNA from 576 PTB and 598 term controls (38-41 weeks) from two independent Caucasian populations (Australian/Danish) were genotyped using high-density SNP arrays. Genotypes were imputed for 2.4 million SNPs. 104 maternal SNPs were identified in the PTBGene database from studies in Caucasians. Univariate logistic regression was performed on both cohorts, including the first two principal components to account for population stratification.
Results: 72 SNPs were present in both PTBGene and the PGP genotype data. Of the 13 positive associations in PTBGene data, two were associated with early PTB in the PGP: rs1081394 in MTTR in the Australian cohort (MAF=0.47, p=0.03, OR=1.32) and rs1979277 in SHMT1 in the Danish cohort (MAF=0.32, p=0.04, OR=1.32). Of the 62 SNPs with no association with PTB in PTBGene, four were associated with early PTB in the PGP samples: two in the Australian cohort [rs1800629 in TNF (MAF=0.17, p=0.04, OR=1.42) and rs4813725 in SLC23A2 (MAF=0.33, p=0.01, OR=0.68)] and two in the Danish cohort [rs590368 in TNFRSF1B (MAF=0.39, p=0.02, OR=0.69) and rs769178 in TNF (MAF=0.04, p=0.01, OR=0.46)]. Genetic variants associated with early PTB in PGP were all protective for the risk of PTB. Simultaneous modelling of three SNPs in each of the cohorts demonstrates protective effect with increasing number of minor alleles (Australian cohort p=0.01, Danish Cohort p=2.16x10-4).
Conclusion: Under powered candidate gene studies have limitations in identifying genetic variants associated with PTB. PGP demonstrates a role for carefully phenotyped samples in adequately sized studies, especially when multiple SNPs are considered simultaneously using new analytic approaches.

T-177

High Cortisol (Stress) Levels during Pregnancy Are Correlated with Low Levels of 25 (OH) Vitamin D. Chander P Arora,[1,2] Payush Chatta,[1] Calvin J Hobel.[1,2] [1]*Ob-Gyn, Cedars-Sinai Medical Center, Los Angeles, CA, USA;* [2]*Ob-Gyn, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA.*
OBJECTIVE: To identify the association between circulating levels of vitamin D and a stress hormone, cortisol during pregnancy.
HYPOTHESIS: Deficiency of vitamin D (a failure of the body to provide an approriate antibacterial peptide (cathelicidine) to counter an inflammatory response) during pregnancy leads to a high cortisol anti-inflammatory response.
STUDY DESIGN: In a behavior in pregnancy study, 524 ethnically diverse women were followed up at T1 (18-20 weeks), T2 (28-30 weeks) and T3 (34-36weeks), samples collected at each stage. Cortisol levels were used as the indicator of low or high levels of stress. Caucasian subjects were categorized into low and high cortisol group and plasma samples analyzed for circulating levels of 25(OH) D.
RESULTS: Out of the Caucasean population with complete sample sets, 34 subjects were identified as the low cortisol group (cortisol levels <50 ng/ ml at T1) and 31 as the high cortisol group (cortisol levels > 150 ng/ml at T1). Cortisol levels at T2 followed the same pattern in both groups(115.9ng/ml in low cortisol group vs 209.5 ng/ml in high cortisol group) and at T3 (59.5 ng/ml in low cortisol group vs 158.4 in high cortisol group). At T1, the levels of vitamin D were sufficient (69.2 nmol/l) in low cortisol group whereas the levels were insufficient (35.7nmol/l) in high cortisol group. Subsequent visits also indicated higher levels of 25(OH) D in low cortisol cases compared to the high cortisol group (T2: 35.4 nmol/l vs 27nmol /l; T3: 43.5nmol/l vs 29.8nmol /l).
CONCLUSIONS: Vitamin D deficiency or even insufficiency may be an unrecognized cause of an exaggerated inflammatory response and a counter regulatory stress response during pregnancy. There appears to be a primary resilience response in the low cortisol group during late pregnancy (34-36 weeks) and less so in the high cortisol group. The reflected resilience may be genetic, environmental or both. This relationship has not been previously recognized and may be associated with poor pregnancy outcome.

T-178

A Quantitative Analysis of Changes in Ion Channel Expression in the Pregnant Heart. Sarah Crimmins,[1] Qinlian Zhou,[2] Glenna CL Bett.[1,2] [1]*Gynecology-Obstetrics, SUNY, University at Buffalo, Buffalo, NY, USA;* [2]*Physiology and Biophysics, SUNY, University at Buffalo, Buffalo, NY, USA.*
Objective:
Although sex differences have been clearly identified in the cardiac EKG for more than a century, the molecular bases of these differences are well not described. Even less well understood is the molecular basis of the adaptation

of the female heart to the physiological remodeling associated with pregnancy. We therefore quantified differences in expression level of mRNA of cardiac ion channels between cycling female, ovariectomized female, pregnant female, and male hearts.

Design: Left ventricular free wall was excised from female cycling (n=8) and pregnant (n=6) ovariectomized (n = 8) and male (n=10) mice (C57BL). mRNA was extracted (Trizol, Invitrogen and RNAeasy, Qiagen) and converted to cDNA (First strand synthesis, SABiosicences). We used quantitative real time PCR (SYBR Green in an iQ icycler, Biorad) to determine changes in relative mRNA expression in the mouse left ventricle. Data were analyzed using ANOVA ($p<0.05$ was regarded as significant).

Results: Significant differences in mRNA expression were observed in the several channels, including those underlying excitatory currents (I_{Na}) as well as the channels underlying repolarizing currents ($I_{to,}$ $I_{Kr,}$ I_{Ks}). Significant changes in expression were divided on the basis of two criteria: predominantly genomically sex dependent changes (i.e., no difference in expression between OVX, pregnant, and female, but all three significantly different vs. male) and predominantly hormonally regulated (i.e., both OVX and male significantly different vs. female and pregnant). Some of the observed changes in pregnancy (e.g., reduction in the relative expression of repolarizing currents) were potentially pro-arrhythmic.

Conclusions: There are significant differences in ion channel expression between female, OVX, pregnant and male hearts. Our analysis indicates that there are significant genomically regulated differences between female and male hearts, as well as hormonally regulated differences. During pregnancy we noted changes which were potentially pro-arrhythmic in a normal heart. However, the pregnant heart has to adapt to a major change in pump load, so these changes may reflect an adaptation to the altered physiology of pregnancy.

T-179

Maternal Cerebral Autoregulatory Capacity Is Diminished during Sheep Pregnancy. Sabine Bischoff,[1] Rene Schiffner,[2] Florian Rakers,[3] Sven Rupprecht,[3] Harald Schubert,[1] Matthias Schwab.[3] *[1]Inst. of Lab Animal Sciences and Welfare, Friedrich Schiller Univ, Jena, Germany; [2]Accident and Emergency Dept., Friedrich Schiller Univ, Jena, Germany; [3]Dept. of Neurology, Friedrich Schiller Univ, Jena, Germany.*

Cerebral vascular tone is decreased during pregnancy leading to a cerebral blood flow (CBF) increase of about 40% (Cipolla, J Appl Physiol 2011). Hemorrhage is a major complication at birth, e.g. due to placental abruption. Functional outcome of severe hemorrhage is mainly limited by cerebral damage due to an insufficient cerebral blood supply (Kudo, Crit Care Med 2006).

Aim: To examine whether pregnancy affects regulation of CBF during severe hemorrhage.

Methods: Five non-pregnant and pregnant sheep were instrumented with femoral arterial and venous catheters under general isoflurane anaesthesia. Single fiber Laser Doppler flow probes (400m diameter, Moor Instr.) were introduced into the cerebral cortex and subcortex (thalamus) following trepanation to monitor capillary CBF changes continuously. Controlled severe hemorrhage was induced by withdrawing 40% of the estimated total blood volume (7% of total body weight).

Results: In non-pregnant sheep, hemorrhage induced a delayed decrease of CBF that was especially pronounced in the subcortex reflecting cerebral autoregulation (Fig. 1, $p<0.05$). In pregnant sheep, the CBF decreased faster in the cortex and subcortex (Fig. 1, $p<0.05$). The maximum cortical and subcortical CBF decrease was more pronounced in pregnant compared to non-pregnant sheep ($p<0.05$).

Conclusions: In pregnancy, autoregulatory function and maintenance of CBF is diminished in the cerebral cortex and subcortex. This is most likely due to a decreased cerebral vascular tone that diminishes the cerebral autoregulatory reserve. In spite of increased baseline CBF (Cipolla, J Appl Physiol 2011), specific vigilance to maintain maternal cerebral blood supply during severe hemorrhage in pregnancy is necessary to prevent cerebral damage.

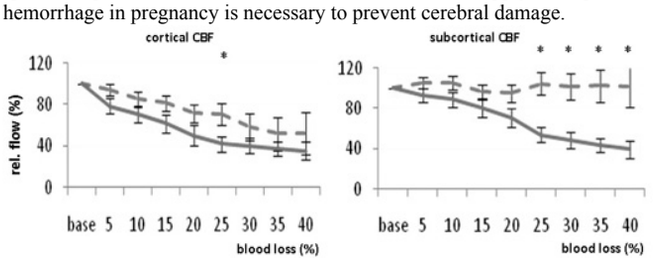


Fig. 1. CBF changes during severe hemorrhage in non-pregnant (dotted line) and pregnant sheep (continuous line). MW±SEM, *$p<0.05$

T-180

Digital Thermal Monitoring of Vascular Function in Pregnancy. Autumn Broady, Kioka Hamada, Janet M Burlingame. *Obstetrics, Gynecology and Womens Health, John A. Burns School of Medicine, University of Hawaii.*

OBJECTIVE: To establish normative data for Digital Thermal Monitoring (DTM) during pregnancy and compare these with measurements from pregnant women with hypertensive disorders and with historical nonpregnant controls.

STUDY DESIGN: Vascular reactivity index was measured by DTM temperature rebound (TR) in pregnant women with the Endothelix VENDYS DTM system at four time points (18-24 and 30-36 weeks gestation and at 4-10 and 16-22 weeks postpartum). Inclusion criteria were pregnant women with and without hypertensive disorders, 18 to 45 years of age and a singleton pregnancy. Exclusion criteria for controls were diabetes, lupus and thyroid disease.

RESULTS: 52 women were recruited (38 controls and 8 with hypertensive disorders). Mean maternal age was 28.3 years (SD = 6.4 years) with a mean body mass index of 30.3 (SD = 6.8). Study participant ethnicity was mixed with predominance of Asians and Pacific Islanders. A total of 84 DTM tests were performed during pregnancy: 43 at time point 1 and 41 at time point 2. 42 DTM tests were performed postpartum: 28 at time point 3 and 15 at time point 4. 12 (14%) DTM tests were invalid. Mean TR values and standard deviations were similar between control and hypertensive patients in both antepartum and postpartum time points (table I).

Table I. TR values and standard deviations in hypertensive patients versus controls at 4 time points

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group	**18-24 weeks gestation (n = 4)**		**30-36 weeks gestation (n = 8)**		**4-10 weeks postpartum (n = 6)**		**16-22 weeks postpartum (n = 2)**	
All Hypertension	2.5	0.6	2.7	0.6	2.9	0.7	2.8	0.3
	(n = 2)		(n = 5)		(n = 3)		(n = 0)	
Preeclampsia only	2.9	0.5	3.0	0.6	3.2	1.0		
	(n = 34)		(n = 32)		(n = 19)		(n = 10)	
Control	2.7	0.6	2.5	0.5	2.9	0.6	2.9	1.0

Mean TR values in healthy pregnant controls differed significantly from historical non-pregnant controls (table II).

Table II: TR values and standard deviations in pregnant controls versus non-pregnant historical controls

Control Group	Group Type	Number per group	Mean age (years)	Mean TR	SD	*P* Value (95% CI)
Current Study	Pregnant Women	38	28	2.7	0.6	
Historical Non-pregnant Control Study #1: Ahmadi et al 2009; 6:431-9	60% Women	78	56	1.6	0.15	< 0.0001 (1.23 to 0.95)
Historical Non-pregnant Control Study #2: Ahmadi et al 2009; 25: 725-38	32% Women	28	61	1.05	1.26	< 0.0001 (2.14 to 1.16)
Historical Non-pregnant Control Study #3: Ahmadi et al 2010; 144(1): 163-65	33% Women	214	56	1.68	0.25	< 0.0001 (1.14 to 0.90)

CONCLUSION: This is the first time DTM has been studied in pregnancy. There was no significant difference in the TR values between controls and pregnant women with hypertensive disorders at any time point in this pilot study. There was a significant increase in the TR in the pregnant control group versus historical non-pregnant controls. This is consistent with vascular and perfusion changes that occur in pregnancy.

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Maternal Running during Pregnancy Improves Glucose Disposal in Obese Mice. Lindsay G Carter, Kristen M Platt, Christine M Tobia, Kevin J Pearson. *Graduate Center for Nutritional Science, University of Kentucky, Lexington, KY, USA.*

Gestational diabetes mellitus (GDM), characterized by poor glucose regulation during pregnancy, occurs in approximately 2 – 10% of pregnancies and causes complications for both mother and fetus. All pregnant women are susceptible to the development GDM, but obese women are at particularly high risk. Two drugs used to treat GDM, glyburide and metformin, have both been shown to cross to the placenta posing a risk to the fetus, making the search for alternative treatments more pressing. Physical activity has been suggested as a non-pharmacological intervention to improve glucose regulation in patients with GDM. To confirm this finding in mice, we hypothesized that voluntary wheel running would improve glucose disposal following a glucose challenge in obese pregnant dams. Female mice were separated into standard diet (SD) and high fat (HF) fed cohorts for 2 weeks. HF-fed mice had increased body weight and impaired glucose disposal compared to SD-fed dams. SD and HF-fed mice were then separated into cages ± running wheels and mated. Analyses were performed on the pregnant mice. Exercise in HF-fed mice significantly decreased body weight, fat mass, and improved glucose disposal between 10-14 days of gestation compared to sedentary HF-fed mice ($p < 0.05$). Glucose disposal in HF-fed dams with access to running wheels was indistinguishable

from dams fed a SD. These results show that exercise can improve glucose regulation in a diet-induced obesity model, and future studies will explore the protective mechanism behind this benefit.

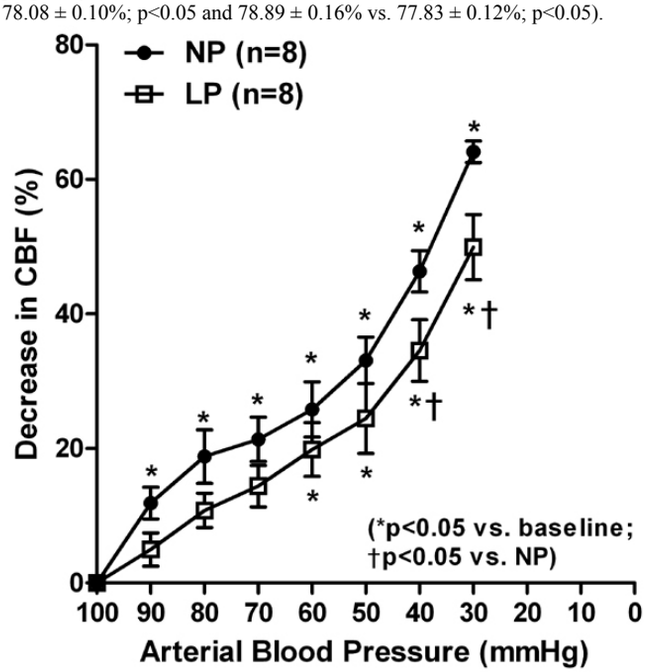
T-182

Pregnancy Improves Cerebral Blood Flow Autoregulation in the Posterior Brain Region during Acute Hypotension. Siu-Lung Chan, Marilyn J Cipolla. *Neurology, Ob/Gyn & Repro Sci, University of Vermont, Burlington, VT, USA.*

Objective: Acute hypotension, such as during hemorrhage or anti-hypertensive treatment, can cause brain ischemia and infarction; however, the impact of pregnancy on cerebral blood flow autoregulation (CBFAR) in response to an acute decrease in pressure is unknown. Thus, we compared CBFAR in response to blood pressure lowering in anterior and posterior brain regions in NP and LP rats. Our second goal was to compare brain edema formation in these animals as a marker of brain injury.

Methods: Female Sprague Dawley NP and LP (E19-21) rats (n=8/group) were anesthetized with chloral hydrate (200 mg/kg) and mechanically ventilated to maintain blood gases. Laser Doppler probes were placed over the anterior and posterior brain regions to measure CBF simultaneously. Systemic blood pressure was decreased by blood withdrawal from a femoral artery catheter from 100 to 30 mmHg and CBF was recorded. Water content of the anterior and posterior brain regions was compared by wet:dry weights.

Results: CBFAR in response to acute hypotension was more effective in the anterior vs. posterior brain region in NP rats. The pressure at which CBF decreased significantly from baseline was 50 mmHg in anterior vs. 90 mmHg in posterior region ($p<0.05$). There was no difference in CBFAR between regions in LP animals. However, compared to NP, pregnancy improved CBFAR in the posterior region such that the pressure at which CBF decreased compared to baseline was 60 mmHg in NP vs. 90 mmHg; $p<0.05$ (Figure). This effect was absent in the anterior brain region (not shown). Brain water content was significantly increased in the anterior vs. posterior brain region regardless of pregnancy (% water for NP and LP in anterior vs. posterior: 79.17 ± 0.08 vs. 78.08 ± 0.10%; $p<0.05$ and 78.89 ± 0.16% vs. 77.83 ± 0.12%; $p<0.05$).



Conclusions: Pregnancy improves CBFAR in response to acute hypotension in the posterior brain region, but not anterior. The increase in water content in the anterior brain region appears not to relate to hypotension or pregnancy.

T-183

An Enhanced Myogenic Vasodilatory Response to Hypotension in Posterior Cerebral Arteries of Pregnant Rats Is Nitric Oxide Dependent. Abbie C Chapman, Siu-Lung Chan, Marilyn J Cipolla. *Neurology, Ob/Gyn & Repro Sci, University of Vermont, Burlington, VT, USA.*

Introduction:

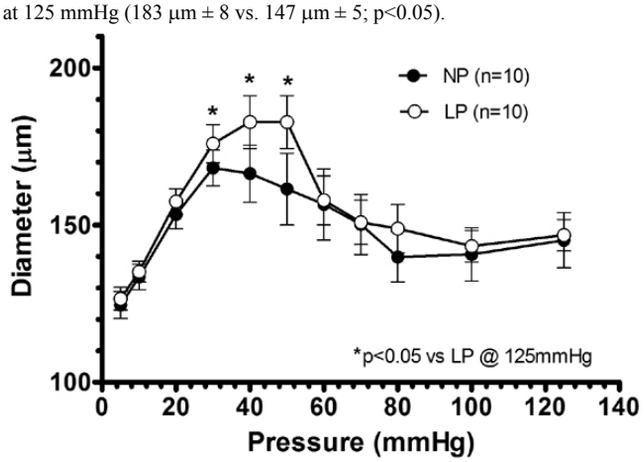
Cerebral blood flow autoregulation (CBFAR) functions to maintain constant blood supply to the brain despite fluctuations in blood pressure (BP). The myogenic vasodilation in response to hypotension is a critical component of CBFAR. In the present study, we investigated the influence of pregnancy on myogenic vasodilation and the autoregulatory response to hypotension and hemorrhage.

Methods:

Posterior cerebral arteries (PCAs) from nonpregnant (NP) and late-pregnant (LP, E20) SD rats were dissected and cannulated in an arteriograph chamber. Myogenic vasodilation was measured in the absence (n=10/group) or presence of the NOS inhibitor N^{ω}–nitro-L-arginine (L-NNA, 0.1mmol, n=7/group) by decreasing pressure from 125 to 5 mmHg and recording luminal diameter. Autoregulation in response to hemorrhagic hypotension was measured *in-vivo* in NP and LP rats (n=8/group) anesthetized with chloral hydrate and ventilated to maintain blood gases. CBF in the PCA territory was measured using laser Doppler.

Results:

PCAs from NP and LP rats developed similar myogenic tone at 125 mmHg (33 % ± 3 and 34 % ± 2; ns). When pressure was decreased, PCAs from LP animals dilated becoming significantly larger at 50 mmHg vs. starting diameter at 125 mmHg (183 μm ± 8 vs. 147 μm ± 5; $p<0.05$).



The dilation of PCAs from LP animals was prevented by L-NNA treatment (not shown). In contrast, PCAs from NP animals dilated less (161 μm ± 11 at 50 mmHg vs. 145 μm ± 9 at 125 mmHg; ns), and L-NNA treatment did not affect myogenic vasodilation. *In-vivo* when BP was lowered by hemorrhage the change in CBF became significant vs. baseline at 90 mmHg in NP animals; however, BP decreased to 60 mmHg before CBF was different vs. baseline in LP animals, suggesting CBFAR to decreased pressure is more stable during pregnancy.

Conclusion:

These results suggest there is an enhanced myogenic vasodilatory response to decreased intraluminal pressure in the pregnant state that is due to NO. This potential role for NO in pregnancy may promote greater effectiveness of CBFAR during hypotension and hemorrhage.

T-184

Effect of Elevated Maternal Testosterone during Pregnancy on Mammary Gland Development and Function. Vijayakumar Chinnathambi, Meena Balakrishnan, Chandra Yallampalli, K Sathishkumar. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*

Intrauterine growth restriction (IUGR) and accelerated postnatal growth is a risk factor for adult diseases. Studies show that elevated maternal testosterone levels during pregnancy leads to IUGR and low birth weight offspring that remain smaller during early postnatal stages but exhibit catch-up growth during later life. An adequate development of the mammary gland and effective delivery of milk to the suckling offspring are essential for normal postnatal growth. We examined whether elevated maternal testosterone during pregnancy impairs mammary gland development and function by causing reduced expression of nutrient transporters and milk delivery to pups that may restrain early postnatal growth.

Methods: Pregnant rats were exposed to testosterone propionate (TP, 0.5 mg/kg) in sesame oil by daily subcutaneous injection from gestational days (GD) 15–19 and control rats received vehicle only. Mammary gland was collected on GD20 and lactation day (LD) 14 to asses mRNA transcripts of nutrient transporters for glucose (*glut1, glut8* & *sglt1*), fatty acid (*fatp1, fatp4* & *fabp3*) and L-carnitine (*octn1, 2* & *3*). Milk intake of individual pups was measured on postnatal day 6, 10 and 14.

Results: The placental weight and pup weight were significantly lower in TP group compared to control consistent with our previous reports. The mRNA

expression of glucose transporters, *glut1 & 8* in the mammary gland did not differ between control and TP at GD20 and LD14, while only secondary active Na^+/glucose transporter (*sglt1*) was significantly lower in TP at GD20 and LD14. The fatty acid binding proteins (*fabp3*) and its transporter (*fatp4*) did not statistically differ between groups at GD20 and LD14 whereas, *fatp1* was significantly lower in TP group at GD20 but not at LD14. L-carnitine transporters, *octn2 & 3* were not altered in TP compared to controls but *octn1* was significantly lower at both GD20 and LD14 in TP group. The milk intake by male and female pups in TP litter was similar to that in controls at postnatal day 6, 10 and 14.

Conclusions: This study shows that elevated maternal testosterone levels has marginal effect on mammary development, particularly on the nutrient transporters, and does not affect milk production or consumption by pups. Further studies examining the nutritional content of milk may help understand if maternal testosterone excess affects milk quality.

T-185

A Longitudinal Study of Skin Barrier Function through Pregnancy and the Early Postnatal Period. Aine Gallagher,[1] Louise C Kenny,[1] Jonathan O'B Hourihane.[2] [1]*Anu Research Centre, University College Cork, Ireland;* [2]*Department of Paediatrics and Child Health, Cork University Hospital, Ireland.*

Background

Pregnancy is a time of profound physiological change, affecting all organs in the body. The skin, the largest organ, may be affected in various ways by pregnancy. 60-80% of women experience eczema for the first time in pregnancy. Eczema is associated with defective skin barrier function with increased transepidermal water loss (TEWL).

Aims:

The characterization of skin barrier function in pregnancy and the postnatal period using non-invasive methods.

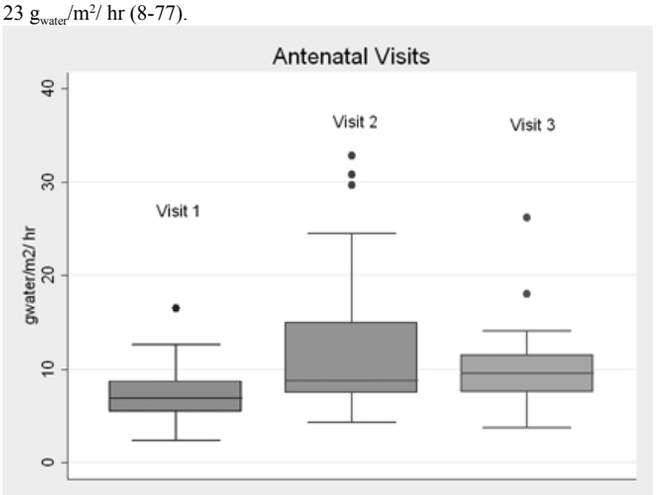
Methods:

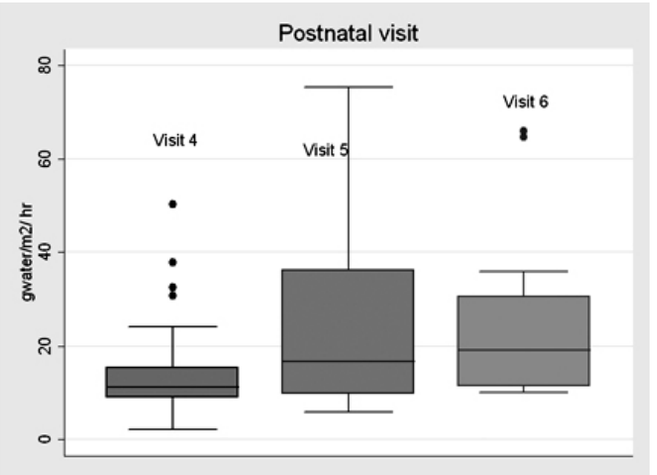
TEWL was measured longitudinally in a cohort of 52 low-risk primagravid women. TEWL was measured using the hydrometer which is based on the vapour pressure gradient estimation. The probe was held in place for 30 seconds per measurement until a steady TEWL value was established. The average of three such values was recorded after each visit.

Measurements were taken three times in the antenatal period: at 19-21 weeks (Visit 1), 27-32 weeks (V2) and 36 weeks (V3) weeks and three times in the postnatal period: on day 1-2 (V4), at 2 months (V5) and 6 months post delivery (V6).

Results:

41 women had a complete set of three antenatal measurements taken and of these 39 women had a complete set of six measurements taken.The mean of each timepoint was: Visit 1 (V1): 9 g_{water}/m^2/ hr (range 2-16), V2: 12 g_{water}/m^2/ hr (4-33), V3: 11g_{water}/m^2/ hr (4-52). The mean value of each postnatal timepoint was: V4: 14 g_{water}/m^2/ hr (2-50), V5: 20 g_{water}/m^2/ hr (6-75) and V6: 23 g_{water}/m^2/ hr (8-77).





Conclusion:

To our knowledge, this is first study to investigate skin barrier function as represented by TEWL during pregnancy. TEWL remains stable during pregnancy and is higher in the postpartum period. The reason for this postpartum rise is unknown and warrants further study.

T-186

The Prevalence of Metabolic Syndrome Following Preeclamptic Pregnancy Does Not Change within 5 Years. Wieteke M Heidema, Ralph R Scholten, Fred K Lotgering, Marc EA Spaanderman. *Obstetrics & Gynecology, Radboud University Nijmegen Medical Centre, Netherlands.*

Introduction

Preeclampsia is associated with cardiovascular disease later in life. In the first year after gestation, formerly preeclamptic women have more often cardiovascular risk factors consistent with the metabolic syndrome (MetS). MetS after preeclamptic pregnancy may reflect a preexisting condition, or a direct post pregnancy effect. We hypothesized that the presence of MetS after preeclampsia is pregnancy-independent and will not change within 5 years following that pregnancy.

Methods

We analyzed, 6-12 months post partum (pp) and again 5 years later the prevalence of constituents of MetS (WHO-criteria; hyperinsulinemia and two or more of the following features: obesity, dyslipidemia, hypertension or proteinuria) in 140 formerly preeclamptic (ACOG-criteria) women. We assessed fasting lipids, glucose and insulin and 24 hours urinary protein and creatinin output. We measured height and weight to calculate BMI. Resting blood pressure was assessed as the median of 9 consecutive oscillometric measurements. Groups were compared using Chi square testing ($P<0.05$).

Results

The presence or absence of MetS was little affected by time (Table 1). The prevalence of obesity and insulin resistance increased over time. Two thirds (67%) of formerly preeclamptic women had MetS both at 6-12 months and 5 years post partum and only 7% developed MetS over time (Table 2). Hyperinsulinemia or dyslipidemia 6 to 12 months post-partum raised the odds on remote MetS (Table 3).

Table 1

	6-12 months pp	5 years pp	p-value
metabolic syndrome	15 (11%)	19 (14%)	0.29
hyperinsulinemia	61 (44%)	78 (55%)	0.03
obesity	22 (16%)	32 (23%)	0.001
dyslipidemia	25 (18%)	23 (16%)	0.62
hypertension	34 (24%)	42 (30%)	0.07
proteinuria	8 (6%)	3 (2 %)	0.10

Table 2

metabolic syndrome	present 5 years pp	absent 5 years pp
present 6-12 months pp	67%	33%
absent 6-12 months pp	7%	93%

Table 3

Factor present 6-12 months pp	MetS 5 years pp	95% CI
	aOR	
hyperinsulinemia	**5.0**	[1.0-24.7]
obesity	4.0	[0.9-17.3]
dyslipidemia	**12.9**	[3.1-54.5]
hypertension	3.6	[0.9-14.9]
proteinuria	1.8	[0.2-18.2]

Conclusion
The prevalence of MetS is unaffected by time during the first 5 years after preeclamptic pregnancy. Screening within one year following preeclamptic pregnancy provides a good indication of the presence or absence of MetS.

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Longitudinal Study of Metabolomic Changes in Plasma and Urine during Normal Pregnancy. Richard P Horgan,[1] Warwick B Dunn,[2] Louise C Kenny,[1,4] David I Broadhurst.[3] *[1]Obstetrics & Gynaecology, University College Cork, Ireland; [2]Centre for Integrative Systems Biology and School of Chemistry, University of Manchester, United Kingdom; [3]Medicine, University of Alberta, Edmonton, Canada; [4]The SCOPE Consortium, .*
Background: The controlled regulation of metabolic interactions between mother, placenta and fetus is fundamental to the development of a healthy baby and in maintaining a healthy state of homeostasis in the mother. The recognition of abnormal events in pregnancy demands a clear understanding of the normal processes of maternal adaptation.
The study of biological systems in a holistic manner (systems biology) is a logical approach to provide a thorough understanding of underlying biological processes. Metabolic profiling is a powerful systems biology strategy for investigating the metabolites present in a cell, tissue or organism.
Aim: To characterize changes in the metabolic profile of plasma and urine taken from normal pregnant women at various time-points during pregnancy and postnatally. In addition, temporal correlations between metabolites were investigated to elucidate the interaction of sensitive metabolites throughout gestation.
Methods: Urine and plasma samples from 30 'normal' nulliparous women were collected at 8 time points. All women were participants in the SCreening fOr Pregnancy Endpoints (SCOPE) study. All samples were analysed using mass spectrometry. Univariate and multivariate statistical and hierarchical cluster analyses (HCA) were performed.
Results: There were significant changes in both the urine and plasma metabolome over the gestational period. 42 metabolite peaks (35 chemically identified) showed significant change between urine samples at different time-points. HCA revealed four clusters of clearly differing metabolite trajectories. 690 features (183 were putatively identified as 'unique' endogenous metabolites) showed significant change between plasma samples at different time-points. HCA revealed five clusters of clearly differing metabolite trajectories. Amino acid, carnitine, phospholipid, vitamin D and energy metabolism were of particular interest and will be discussed fully.
Conclusion: The results demonstrated that pregnancy has a significant impact on the metabolomic profile of urine and plasma. The results also show the temporal relationship between metabolites and the individual biological variation of normal pregnant women.
This work was funded by the Health Research Board in Ireland (CRT/2007/5).

T-188

Has Increased Clinical Experience with Methotrexate Reduced the Direct Costs of Medical Management of Ectopic Pregnancy Compared to Surgery? Andrew W Horne,[1] Daniel T Westaby,[1] Olivia Wu,[2] Stephen Tong,[3] Hilary OD Critchley,[1] W Colin Duncan.[1] *[1]MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom; [2]Health Economics and Health Technology Assessment Unit, University of Glasgow, United Kingdom; [3]Department of Obstetrics and Gynaecology, University of Melbourne, Australia.*
Laparoscopic surgery and methotrexate can both be safely used to treat small unruptured ectopic pregnancies (EP) with hCG concentrations <3000 IU/L. Although medical management of EP is generally considered to be more cost-effective than surgery, a meta-analysis performed in 1999 concluded that methotrexate is only less expensive than surgery in women whose serum hCG concentrations were <1500 IU/l. We hypothesised that further clinical experience with methotrexate, and the increased use of guideline-based management protocols, has made medical management less expensive than surgery in all women with hCG concentrations <3000 IU/L. A retrospective cost analysis was conducted on women treated either medically or surgically for EP at a large teaching hospital between January 2010 and February 2011. Management criteria and eligibility for methotrexate followed national UK guidelines. Patients were categorised as follows: those who were eligible for medical or surgical management but opted for methotrexate (n=48), those who chose surgery but were eligible for methotrexate (n=20), and those who required surgery because they were not eligible for methotrexate (n=59). Of those who elected to have medical management, methotrexate treatment was £1179 (CI 819-1550) cheaper than elected surgical management reflecting potential savings per patient. However, there were no significant savings over surgery among those who elected to have methotrexate with hCG concentrations >1500 IU/L due to a higher occurrence of treatment failure. Although medical management of EP remains significantly less expensive than surgical management for patients with hCG concentrations <1500 IU/L, our data support an ongoing and unmet economic need for biomarkers predictive of methotrexate failure or for better medical treatments for EP with hCG concentrations >1500IU/L.

T-189

Previous Pregnancy Loss Has an Adverse Impact on Cognitive, Behavioural and Emotional Well-Being in Pregnancy. Fergus P McCarthy,[1] Ali S Khashan,[1] Robyn A North,[2] Philip N Baker,[3] Gus Dekker,[4] Lucilla Poston,[2] Keelin O'Donoghue,[1] Louise C Kenny.[1] *[1]The Anu Research Centre, University College Cork, Cork, Ireland; [2]Women's Health Academic Centre, King's College, London, United Kingdom; [3]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada; [4]Obstetrics and Gynecology, Adelaide University, Adelaide, Australia.*
Objectives
Previous miscarriage is associated with significant depression and anxiety. This study investigated 1) the association between women with previous miscarriages and altered cognitive, behavioural and emotional well-being in pregnancy 2) whether the magnitude of any observed changes were related to the number of previous miscarriages
Methods
This prospective cohort study consisted of 3531 nulliparous women recruited in the Screening for Pregnancy Endpoints (SCOPE) study.[1] Women with 1 and 2 or 3 previous miscarriages were compared with women who had no previous miscarriages. Outcomes included Edinburgh Postnatal Depression Scale (EPDS) score (range 0-30), Perceived stress scale score (PSS, range 0-30), Short form State-Trait Anxiety Inventory (STAI) score (range 6-24) and limiting behavioural response to pregnancy score (range 0-20).
Results
2624 women had no previous miscarriage (reference group), 691 had 1 previous miscarriage and 216 had 2 or 3 miscarriages. The estimates of the associations are summarized in Table 1.

Table 1

	1 miscarriage (n=691), adjusted mean difference (95% CI)	2 or 3 miscarriages (n=216), adjusted mean difference (95% CI)
Outcomes		
EPDS		
15 weeks	0.6(0.2,1.0)	1.8(1.1,2.4)
20 weeks	0.6(0.2,1.0)	1.2(0.6,1.9)
PSS		
15 weeks	0.7(0.2,1.2)	1.6(0.8,2.5)
20 weeks	0.7(0.2,1.3)	1.3(0.4,2.2)
STAI		
15 weeks	1.6(0.7,2.6)	1.8(0.2,3.4)
20 weeks	1.0(0.1,1.9)	1.2(-0.3,2.8)
Limiting response		
15 weeks	0.4(0.1,0.7)	0.9(0.4,1.4)
20 weeks	0.4(0.1,0.7)	0.9(0.4,1.4)

Adjusted for maternal age, smoking, alcohol, ethnic origin, BMI and SCOPE centre
Conclusion
Women with previous miscarriages have increased cognitive, behavioural and psychological ill-health in pregnancy. The magnitude of these changes are related to the number of previous miscarriages. These changes persist in the second trimester. Final analysis will include data from the final cohort of 5690 women.
References
1. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. North et al. BMJ. 2011;342:d1875. doi: 10.1136/bmj.d1875.PMID:21474517

T-190

Limitations of Clinical Risk Screening for Gestational Diabetes Mellitus. Nicolai Murphy, Ali Khashan, Louise C Kenny, Mairead O'Riordan. *Anu Research Centre, Dept. Obstetrics and Gynaecology, University College Cork, Cork, Ireland.*
Background: Gestational Diabetes Mellitus (GDM) affects 2-10% of all pregnancies and is associated with increased maternal and neonatal morbidity. There is intense debate as to whether to adopt universal screening in place of screening based on clinical risk factors. Universal screening has higher detection rates but significant economic implications. Screening based on

clinical risk factors is reported to have widely varying detection rates. It has been suggested that this may be due in part to the incorrect application of screening guidelines.[1]

Aims: To investigate compliance with local guidelines for GDM screening in prospective observational cohort of nulliparous, low risk women participating in the SCOPE (Screening for Pregnancy Endpoints) study.

Methods: Local guidelines for screening included family history of diabetes in a first degree relative, BMI ≥30 at booking, previous unexplained stillbirth, recurrent glycosuria, women on long term steroids, previous baby weighing ≥4.5kgs, polycystic ovarian syndrome (PCOS) and polyhydramnios and/or macrosomia in existing pregnancy.

The risk factors assessed in this study include, BMI ≥30 at booking, a first degree relative with a diagnosis of diabetes and a diagnosis of PCOS.

The study cohort consisted of 1792 nulliparous low risk women who participated in the SCOPE study.

Results: Of the 1792 recruited 221 had a BMI ≥30, 215 had a family history of diabetes and 79 had a diagnosis of PCOS. A total of 460 (25.7%) participants had one or more risk factors.

Of the 221 with a BMI ≥30, 53 (24%) were not screened.

Of the 215 with a family history of diabetes, 67 (31.2%) were not screened.

Of the 79 diagnosed with PCOS, 35 (44.3%) were not screened.

Furthermore, women who should have been screened according to the guidelines but were not, had significantly increased risk of Caesarean section compared to women who had no risk factors (OR=1.45; [95% CI: 1.06-1.99]).

Conclusion: Adherence to guidelines for selective clinical risk factor based screening for GDM is poor. This may contribute to the low detection rate associated with risk factor based screening. Furthermore the significant risk of adverse outcomes in the unscreened population with risk factors supports the introduction universal screening.

1. Simmons D et al. Difficulties in the use of risk factors to screen for gestational diabetes mellitus. Diabetes Care 2009;32:e8

T-191

Endothelial Progenitor Cells as Postpartum Cardiovascular Risk Indicators Following Preeclamptic Pregnancies. Malia SQ Murphy,[1] Richard Casselman,[1] Graeme N Smith.[1,2] *[1]Biomedical & Molecular Sciences, Queen's University, Kingston, ON, Canada; [2]Obstetrics & Gynaecology, Kingston General Hospital, Kingston, ON, Canada.*

Introduction: Women with a history of preeclampsia (PE) are at increased lifetime risk for cardiovascular disease. Endothelial progenitor cells (EPC) represent novel biomarkers of vascular health and are implicated as indicators of the endothelial dysfunction characteristic of PE. This study aims to determine differences in EPC levels at delivery in PE pregnancies and whether these differences persist into the postpartum period.

Methods: Circulating CD34+/VEGFR2+/CD45+ and CD133+/VEGFR2+/CD45+ EPCs were determined by flow cytometry in PE and normotensive women at delivery, 2 months and 6 months postpartum. Plasma samples were also collected for analysis of anti-/pro- angiogenic factors, including progenitor mobilization cytokines. Participants with traditional cardiovascular risk factors prior to pregnancy were excluded.

Results: Women following normotensive (n=13) and PE (n=6) pregnancies have been recruited to date. Preliminary results for delivery and 2 months postpartum indicate that levels of EPC subsets identified in monocytic populations are significantly higher following PE pregnancies compared to controls (P<0.05).

CD34+VEGFR-2+CD45+ circulating EPCs in Normotensive and PE women

Control:Term	Control:2 months postpartum	PE:2 months postpartum
0.1037883	0.002925174	0.5417045
0.004482094	0.003114683	2.110560
0.00673242	0.000000	
0.000000	0.01399748	
0.04724941	0.006497867	
0.000000	0.01111451	
	0.000000	
	0.002033636	

P<0.001 for 1wANOVA, crude analysis. PostHoc:Ctrl Term vs Ctrl 2m pp= ns.Ctrl Term vs PE 2m pp= *.Ctrl 2m pp vs PE 2m PP= ***

CD133+VEGFR-2+CD45+ circulating EPCs in Normotensive and PE women

Control: Term	Control: 2 months postpartum	PE: 2 months postpartum
0.01714776	0.000000	0.01619119
0.004543183	0.002867466	2.319043
0.003449227	0.000000	
0.000000	0.01484505	
0.003357169	0.004401699	
0.000000	0.005364375	
	0.000000	
	0.008472243	

**P<0.05 for 1wANOVA, crude analysis. PostHoc:Ctrl Term vs Ctrl 2m pp= ns.Ctrl Term vs PE 2m pp= *.Ctrl 2m pp vs PE 2m PP= *

Plasma samples are currently under analysis.

Conclusions: Preliminary observations indicate EPC levels are elevated in the postpartum period following PE. Longitudinal followup of participants will determine if EPC discrepancies persist and/or progress. Correlation with traditional angiogenic and metabolic risk factors for cardiovascular disease at endpoint will gauge the value of EPCs as a biomarker for premature cardiovascular disease. This study is currently ongoing.

Funded by PSI Foundation.

T-192

The Brain Study: Cognition, Quality of Life and Social Functioning Following Preeclampsia. Ineke R Postma,[1] Henk Groen,[2] Thomas R Easterling,[3] Eleni Z Tsigas,[4] Gerda G Zeeman.[1] *[1]Department of Obstetrics and Gynecology, University of Groningen, University Medical Center Groningen, Netherlands; [2]Department of Epidemiology, University of Groningen, University Medical Center Groningen, Netherlands; [3]Department of Obstetrics & Gynecology, University of Washington, Seattle, USA; [4]Preeclampsia Foundation, , USA.*

OBJECTIVE Preeclampsia (PE) is considered a transient event. However, one-third of formerly preeclamptic women have cerebral white matter lesions. The significance of such lesions is unknown but may be ominous considering the frequently expressed neurocognitive and psychosocial problems following PE, complaints that have largely been ignored or attributed to the stresses of a complicated pregnancy. This study aimed to identify the scope of neurocognitive and psychosocial problems following PE.

METHODS Through website promotion and a mass email all members of the Preeclampsia Foundation were asked to complete 3 web-based questionnaires: the Cognitive Failures Questionnaire (CFQ), the Social Functioning Questionnaire (SFQ) and the abbreviated WHO Quality Of Life questionnaire (WHOQOL-BREF). Participants who experienced PE in the past 20 years were included (cases). Participants were stimulated to ask a friend who had a normotensive pregnancy to complete the questionnaires as well (controls). Women with current or past neurological conditions were excluded. Analysis was done using Mann Whitney U test.

RESULTS A total of 966 cases and 342 controls were included. Mean age was 33.6 and 34.1, mean time since first pregnancy was 5.0 and 5.8 years respectively. Cases scored significantly worse on all 3 questionnaires and more often visited a psychiatrist, currently (17% vs. 9%) or in the past (33% vs. 22%). Women who had eclamptic seizures (n=58) scored worse on the CFQ compared to cases without eclampsia (n=908). All Cronbach's alphas were higher then 0.7.

CONCLUSIONS Women who experienced PE and particularly those with eclampsia, report more cognitive and social problems and worse quality of life. Even though selection bias may be at play this study provides important information about the scope of such problems. Health care providers and patients should be aware of this so that affected women may receive recognition, psychological care and escape from the ignorance of their environment. These findings may stimulate further research relating to the origin as well as management of these important issues.

T-193

Maternal Exercise (MEx) before and in Pregnancy Improves Metabolic Phenotype at Post Natal Day (PND) 110 in Male Rat Offspring (OFF) of Obese Mothers (MO) Fed a High Energy Diet (HED). Luis Reyes,[1] Carlos Ibanez,[1] Magaly Vazquez,[1] Fabiola Cruz-Perez,[1] Claudia J Bautista,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] *[1]Departamento de Biologia de la Reproduccion, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; [2]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

INTRODUCTION: MO in pregnancy has reached epidemic proportions across the world. OFF of MO mothers are predisposed to altered metabolism including glucose (GL) intolerance tolerance and adiposity.

HYPOTHESIS: We hypothesized that MEx prior to and in pregnancy improves male OFF metabolic outcomes.

METHODS. From weaning through pregnancy and lactation rats ate chow (C - 22% protein, 5% fat, 31% polysaccharide (PS), 31% simple sugars (SS), 4% fiber, 4 Kcal/g) or HED (23.5% protein, 20% animal lard, 5% fat, 20.2% PS, 20.2% SS, 5% fiber, 4.9 Kcal/g). Half the C and MO mothers wheel-ran 30 min, 5 times/ week from PND 90 to delivery (CEx and MOEx). All mothers were bred at PND 120 and continued their diet. OFF were weaned on to C. Male OFF (n=8, one per litter) were euthanized at PND 110 and fat depots weighed, body weight (BW), serum leptin, triglycerides (TG), GL, insulin and HOMA measured. Statistics Two way ANOVA (maternal diet and Ex);

RESULTS:

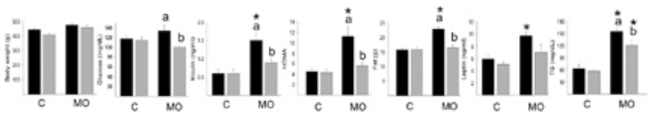


Fig 1 OFF at PND 110: Solid – no Ex, grey – Ex; Data M ± SEM, Different letters significantly different effect of Ex in either C or MO, * C vs. MO for No Ex or Ex *, $p < 0.05$.

CONCLUSIONS: MO had no effect on OFF weight but increased all metabolic variables measured. While Ex had no effect in C it reversed the increases in all MO OFF variables except leptin in the absence of any difference in weight showing the importance of body composition in determining metabolic phenotype.

T-194

Does In Vitro Fertilization Treatment Should Be Considered a Risk Factor for Post Partum Depression. Tomer Singer, Jason Kofinas, Lauren Murphy, Lauren Zakarin, Jack Y Huang, Glenn L Schattman, Dan A Goldschlag, Zev Rosenwaks. *The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA.*

OBJECTIVE: To evaluate the correlation between the mode of conception and the presence of maternal depressive symptoms as expressed by the Edinburgh Postpartum Depressive Scale (EPDS)

DESIGN: Prospective study

SETTING: A post partum unit in a large academic medical center

METHODS: Women giving birth in our medical center were recruited postpartum. Information was obtained from the hospital computerized data base shortly after delivery. Depression status was assessed at 2 days post partum using the Edinburgh Postnatal Depression Scale (EPDS). The study was conducted under an approved institutional IRB

Data was collected between 05.2011-10.2011. Postpartum depression was evaluated using the EPDS. The 10-questions EPDS questionnaire is a valuable and efficient tool for identifying patients at risk for perinatal depression. A score of ≥10 indicates a depressive illness of varying severity. The EPDS scores were correlated with study variables such as maternal age, gravity, parity as well as mode of conception and delivery. Data was analyzed using Statsdirect.

RESULTS: Complete data were obtained from 129 women. 25 patients conceived using In Vitro Fertilization (IVF) treatment and 104 conceived spontaneously. Total of 18 patients (14%) had pathological scores.

IVF patients had a significantly higher EPDS score (5.64 Vs 3.32, P<0.05). Further more, 36% of the IVF group had an abnormal EPDS score compared with 8.6% in the non-IVF group (P<0.05). In our study, IVF patients were 7 years older, delivered 6 days earlier and had a cesarean section rate of 80% compared with 43% in the non-IVF group.

CONCLUSIONS: The results emphasize the importance of evaluating risk factors for postnatal depression and suggest that conceiving using artificial reproductive techniques may play a role in the development of post partum depression. Early identification of potential risk for postpartum depression should include assessment of conception method in addition to psychiatric history.

	IVF	Non IVF	P-Value
N	25	104	
Age	40.52±3.99	33.77±4.73	<0.0001
Gravity	2.44 ±1.55	2.29±1.62	0.5692
Parity	0.6±1	0.69±1	0.3978
Gestational Age	266±18.68	274.43±11.49	0.0152
Prior depression/anxiety	0.15±0.46	0.03±0.19	0.3776
C-Section rate (%)	80	43.26	0.001
Mean EPDS	5.64±4.91	3.32±3.55	0.0268
Abnormal EPDS (%)	36	8.6±	0.0003

T-195

Ten Year, Thirty Year and Life-Time Risk Estimates of Cardiovascular Disease Following a Pregnancy Complicated by Pre-Eclampsia. Graeme N Smih, Jessica Pudwell, the PE-NET. *Obstetrics & Gynecology and Biomedical & Molecular Sciences, Queen's University, Kingston, ON, Canada.*

Objective. To calculate the cardiovascular disease (CVD) risk estimates for women following a pregnancy with or without pre-eclampsia.

Background. The latest American Heart Association's *Evidence-Based Guidelines for the Prevention of CVD in Women* identifies certain pregnancy complications as relevant in the determination of CVD risk in women.

Methods. Women recruited into the Pre-Eclampsia New Emerging Team's prospective longitudinal cohort had their 10-year, 30-year and Lifetime risk estimates for CVD calculated at one year postpartum. Furthermore, the Ontario Government's Better Outcomes Registry Network database for all births was queried to determine the maternal prevalence of traditional cardiovascular risks (CVR) and pregnancy-related CVR indicators.

Results. Complete CVR screening data was obtained from n=118 control women and n=99 pre-eclamptic women in the cohort. 18.2% of women in the pre-eclamptic group and 1.7% of control women (OR 13.08, 95%CI 3.38, 85.5) were identified as having a high 10-year risk, 31.3% of women in the pre-eclamptic group and 5.1% of control women (OR 8.43, 95% CI 3.48, 23.23) were identified as having a high 30-year risk and 41.4% of women in the pre-eclamptic group and 17.8% of control women (OR 3.247, 95%CI 1.76, 6.11) were identified as having a high Lifetime risk for CVD. Between 2005-2009 (n=644,412), 20.58% of women in Ontario had one or more pregnancy-related CVR indicator.

Conclusions. The association of pre-eclampsia and other pregnancy complications with the presence of underlying CVR and the future development of CVD makes pregnancy an early window of opportunity for health preservation and CVD prevention.

T-196

Premature Cardiovascular Aging in Women with SLE. JP Dhar,[1] L Essenmacher,[1] J Ager,[1] L 'Chiodo,[1] D Schultz,[2] A Stark,[3] A Schwartz,[1] L Gregoire,[1] RJ Sokol.[1] *[1]SOM, Wayne State University; [2]SOM, Henry Ford Health Systems; [3]SOM, Geisinger Health Systems.*

Background: Systemic lupus erythematosus (SLE) has peak prevalence in women of childbearing age. The gynecologist is frequently the primary care doctor for pre-menopausal women, making it important to understand health issues in this population. Premature atherosclerosis is known to occur in SLE. Thus, we sought to assess if cardiovascular and metabolic conditions were increased in SLE.

Methods: Charts were reviewed for clinical information on 647 predominantly African American women (71.9%) with SLE, mean age at diagnosis =33.1 years, with 331 being premenopausal (<50yrs) and 292 having severe disease. This study was approved by the Human Investigation Committee at Wayne State University. We did 2 stratifications: 1.) Chi Square analysis to determine if cardiovascular complications (CVC) and metabolic risk factors (MRF) were increased in more severe lupus vs. mild lupus, and 2.) Z test for proportions to calculate risk ratios for premenopausal women (<50 yrs) relative normal females in the same age range. Expected population frequencies were obtained from NHANES.

Results: There was a significant increase in risk ratios for myocardial infarction (MI), transient ischemic attack (TIA), hyperlipidemia (HL), and hypertension (HTN) over the general population, but not for diabetes (DM). In addition, there was no association of prior history of hypertensive complications of pregnancy with any of these variables. Furthermore, MI, TIA, HTN, HL, and DM were significantly increased in severe lupus relative to mild lupus.

Cardiovascular and metabolic complications in SLE

variable	frequency, n (%) in age <50	risk ratio (age <50 vs gen. pop.)	significance for RR, p (Z proportions)	frequency, n(%), in severe disease	significance, p, for increase in severe disease vs. mild
HTN	163 (49.2%)	1.20	<0.001	231 (79.1%)	<0.001
DM	43 (13%)	1.10	NS	68 (23.3%)	<0.01
hyperllpidemia	107 (32.3%)	1.90	<0.001	161 (55.1%)	<0.001
TIA	11 (3.3%)	4.13	<0.001	30 (10.3%)	<0.001
MI	18 (5.4%)	5.4	<0.001	35 (12.0%)	<0.01
total patients	331	N/A	N/A	292	N/A

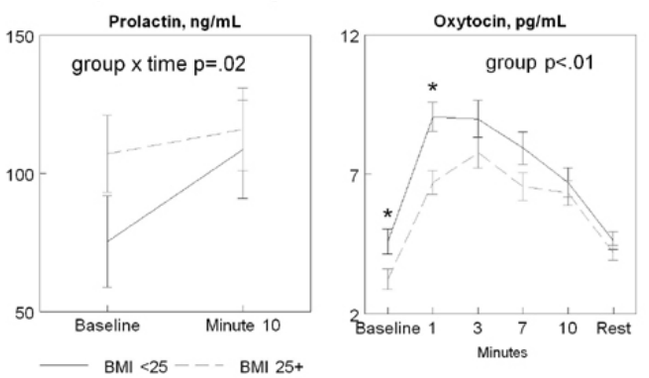
Conclusion: Pre-menopausal women with lupus and those with severe disease have increased frequencies of CVC and MRFs relative to the general population. Few gynecologists are aware of the premature cardiovascular aging in chronic

SLE. These results highlight the importance of recognizing that young women with SLE would benefit from early and aggressive cardiovascular disease prevention regimens.

T-197

Maternal BMI and Neuroendocrine Physiology during Lactation. Alison M Stuebe,[1] Samantha Meltzer-Brody,[2] Karen Grewen.[2] *[1]Obstetrics and Gynecology, UNC School of Medicine; [2]Psychiatry, UNC School of Medicine.*
Background: Obesity is associated with reduced breastfeeding duration and intensity. Rasmussen et al previously reported that overweight maternal BMI was associated with reduced prolactin response to suckling in the first week postpartum. We hypothesized that overweight BMI would be associated with reduced prolactin (PRL) and oxytocin (OT) response in established breastfeeding.
Methods: We conducted a secondary analysis of women participating in a longitudinal study of maternal mood and lactation physiology. Mothers intending to breastfeed were recruited in the third trimester of pregnancy and visited the Maternal Infant Biobehavioral Laboratory at 2 and 8 weeks postpartum for a feeding session. Height and weight were measured and maternal mood was assessed using the Edinburgh Postnatal Depression Scale (EPDS). Maternal blood samples were obtained via antecubital IV at baseline, at minutes 1, 3, 7 and 10 of feeding, and 10 minutes after feeding. We used mixed repeated measures analysis to compare PRL and OT during breastfeeding among normal weight (BMI <25 kg/m2) vs. overweight (BMI 25+ kg/m2) women. Main effect p values <.05 and interaction p values <.10 were considered statistically significant.
Results: Of the 52 women recruited in the 3rd trimester of pregnancy, 42 were breastfeeding at 2 weeks and 37 at 8 weeks. We found no association between maternal BMI and EPDS score or psychiatric history. At 2 weeks, we found no differences in OT or PRL during suckling among overweight (n=28) vs normal weight (N=14) women. At 8 weeks, overweight women (n=22) had lower OT (group p<.01) and diminished PRL change after suckling (group x time p=0.02) compared with normal weight women (n=15), adjusting for parity, breastfeeding intensity, current antidepressant use, and days postpartum (Figure).
Conclusions: We found that maternal overweight was associated with diminished PRL and OT response to suckling at 8 weeks postpartum. These differences may mediate observed associations between maternal obesity and reduced breastfeeding duration and intensity.

Figure: Overweight BMI at 8 wks postpartum is associated with reduced PRL and OT response to breastfeeding.



T-198

Maternal and Fetal Bile Acids in Intrahepatic Cholestasis of Pregnancy. Victoria Geenes,[1] Dominic Lawrance,[1] Anita Lovgren-Sandblom,[2] Lisbet Bethin,[2] Lucy Chappell,[3] Jim Thornton,[4] Jenny Chambers,[1] Vinita Gurung,[4] Peter H Dixon,[1] Hanns-Ulrich Marschall,[5] Catherine Williamson.[1] *[1]Maternal and Fetal Disease Group, IRDB, Imperial College London, United Kingdom; [2]Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden; [3]Division of Women's Health, King's College London, United Kingdom; [4]Maternity Department, City Hospital, Nottingham, United Kingdom; [5]Institute of Medicine, Gothenburg University, Gothemburg, Sweden.*
Background:
Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder of pregnancy characterised by elevated maternal serum bile acids and associated with significant risk of adverse fetal outcomes. The aetiology of fetal complications in ICP remains unclear, but it is hypothesised that bile acids cross the placenta and accumulate in the fetal compartment. However, data from cord serum are limited. This study aimed to establish the maternal and fetal bile acid profiles in normal and cholestatic pregnancy with/without ursodeoxycholic acid (UDCA) treatment.
Methods:
Maternal and cord blood was collected from women with ICP (n=65, 47 treated) and normal controls (n=15) at the time of delivery. 15 serum bile acids were identified and measured by HPLC-MS/MS. Statistical analysis was performed with Mann-Whitney U/ Student T Tests.
Results:
Total bile acids (TBA) were significantly higher in both maternal and cord blood from women with ICP compared to controls, predominantly due to significant increases in the conjugated primary bile acids. Tauro-conjugates were more prevalent than glyco-conjugates. There were no differences in TBA or individual BA in the umbilical artery and vein. Treatment with UDCA reduced TBA in both maternal and cord samples, although only significantly in maternal samples. There was a transplacental gradient from fetus to mother in samples from control pregnancies. This was reversed in ICP and partially corrected by UDCA. Importantly, conjugated UDCA was the predominant bile acid in treated pregnancies and levels of the toxic bile acid, lithocholic acid, were not increased in this group.
Conclusions:
This is the largest study of maternal and fetal bile acids in ICP cases and controls to date. ICP is associated with both qualitative and quantitative changes in maternal and fetal bile acid profiles and there is no evidence for fetal metabolism of the measured bile acids. UDCA reduces TBA in the maternal and fetal compartments and partially corrects the transplacental bile acid gradient.

T-199

A Comparison: The Use of Postoperative Analgesia after Caesarean Section. Diego Garcia Picasso,[1] Julieta Abrile,[2] Orestein Silvia,[2] Vina Agustin,[2] Julia Zollner.[3] *[1]Anaesthetics, General Hospital of Acute Cases "Dr. J. M. Ramos Mejía" - G.C.B.A., Buenos Aires, Argentina; [2]Obstetrics & Gynaecology, General Hospital of Acute Cases "Dr. J. M. Ramos Mejía" - G.C.B.A., Buenos Aires, Argentina; [3]Obstetrics & Gynaecology, Queen Charlotte's and Chelsea Hospital, London, United Kingdom.*
Introduction
Morphine used in the neural axis has proved to be an effective and safe way of postoperative analgesic method after caesarean section (CS).
Objectives
To compare the analgesic effect of 100 mcg of intrathecal morphine with 1 mg of peridural morphine in the first 24 hours after CS. To assess and compare adverse effects of either analgesic method.
Methods
We recruited 200 patients who underwent a CS at our hospital. They were randomized into two groups receiving one dose of either IT (intrathecal, morphine 100 mcg) or EP (Epidural, morphine 1 mg). In addition all groups received 400 mg of ibuprofen every 6 hours and 10 mg of metoclopramide every 8 hours, post-operatively.
The pain intensity was measured according to a verbal numeric scale.
The analgesic satisfaction was assessed after 24 hours according to a scale from 0 to 100. Adverse effects were noted down. The chi-square test was applied for proportion comparison, considering significance at $p < 0.05$.
Results
From the 200 patients, 6.3% showed moderate pain and 2.8% experienced severe pain in the post-operative period. 6.8% had moderate nausea, 1.4% severe, 16.7% moderate pruritus and 2.8% severe. There were no significant difference with respect to pain, nausea or pruritus. There was no profound sedation or respiratory depression. The analgesic satisfaction in both groups was higher than 90 in 95% of the patients.
Conclusion
Both intrathecal morphine and epidural proved to be safe and effective alternatives for postoperative pain control after CS. Both shared a similar profile in regard to analgesic quality and adverse effects.

T-200

Hormone Therapy in Postmenopausal Women Increased Working Memory-Related Brain Activation. Christina Broadwell,[1] Jennifer Y Soung,[2] Peter Casson,[1] Paul A Newhouse,[3] Julie A Dumas.[2] *[1]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Psychiatry, University of Vermont, Burlington, VT, USA; [3]Psychiatry, Vanderbilt University, Nashville, TN, USA.*

Introduction:

Cognitive decline during the menopausal transition is a common concern among women. To date, studies investigating the possible role of hormone therapy in treating these symptoms are conflicting. Prior studies have shown that working memory-related brain activation was sensitive to hormone manipulation in postmenopausal women. No study has yet examined the effect of prior hormone exposure on brain activation during working memory tasks.

Methods:

Twenty-four postmenopausal women were enrolled in a larger study examining the estradiol-cholinergic interaction. Twelve women had a prior history of at least one year of hormone therapy use and 12 women had never taken hormone therapy. We examined functional magnetic resonance imaging (fMRI) activation and working memory performance at the baseline assessment before any estrogen therapy was administered.

Results:

In postmenopausal women with a history of hormone therapy use (range 1-15 years) compared to women who were never users, increased activation was seen in the anterior cingulate and posterior cingulate which are areas normally activated during a working memory task. In addition, increased activation for users relative to never users was seen in the medial temporal lobes and hippocampus, which are regions not often involved in working memory.

Conclusions:

The implication of this increased brain region activation is uncertain at this time. The increased activation for prior users compared to never users may represent a compensatory response to the aging process in general that allows adequate task performance. Alternatively, it may represent the non-specific effect of hormone therapy on modifying brain activity that is unrelated to task performance. In either circumstance, this differential brain activation remains present even one year after discontinuation of use of hormone therapy in postmenopausal women. Further studies will elucidate into this phenomenon.

T-201

Decreased Ovarian Function Is Associated with Central Adiposity. Mamie McLean,[1] Xin Wang,[2] Gordon W Bates,[1] Cora E Lewis,[2] Pamela Schreiner,[3] Barbara Sternfeld,[4] David Siscovick,[5] Melissa Wellons.[1] *[1]Reproductive Endocrinology and Infertility, UAB, Birmingham, AL; [2]Division of Preventative Medicine, UAB, Birmingham, AL; [3]Division of Epidemiology and Community Health, Univ. of Minnesota School of Public Health, Minneapolis, MN; [4]Division of Research, Kaiser Permanente, Oakland, CA; [5]Dept of Epidemiology, Univ. of Washington, Seattle, WA.*

Objective: The perimenopause to menopause transition is associated with gains in abdominal adiposity, potentially related to changes in sex steroid hormones. We hypothesize that earlier changes in ovarian function are also associated with changes in adiposity. We assessed the association between decreased ovarian reserve (DOR) and change in BMI and waist circumference (WC).

Methods: Data came from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a longitudinal population-based multicenter cohort that began in 1985-86. In 2002-03, the CARDIA Women's Study (CWS) measured FSH levels and performed a transvaginal ultrasound (TVUS). Antral follicle count (AFC) by TVUS was defined as the total number of 2-10mm follicles. Main exposure was DOR, defined as AFC <= 4 and FSH >13. The main outcomes were change in BMI and waist circumference (WC) from 2000-01 to 2005-06. ANCOVA was used to assess associations between DOR and outcomes, after controlling for age, race, smoking status, and baseline BMI and WC.

Results: In our sample of 427 premenopausal cycling women, 59 had DOR. In 2000-01, the DOR and non-DOR groups had similar proportions of black women and current smokers (45% vs. 41% p=0.54; 22% vs. 20% p=0.46). In 2000-01, the groups also had similar BMIs and WC (29.3 kg/m ^{2} vs. 28.8kg/m^2; p=0.63 and 86.2cm vs. 85.4cm; p=0.72). At TVUS, the DOR group was older (44.5 yrs vs. 41.8 yrs; p<.001). In 2005-06, the DOR and non-DOR groups had similar BMIs but different WC (31.02kg/m^2 vs. 29.6kg/m^2; p=0.16 and 87.5cm vs. 85.6cm; p=0.04). Over 5 years (2000-01 to 2005-06), there was no significant difference in BMI gain between groups (0.96kg/m^2 vs. 0.20 kg/m^2; p=0.13). But individual DOR women had greater WC gains than non-DOR women (5.65 cm vs. 1.86 cm; p=.002). This difference remained significant after adjustment for age, race, smoking status, and baseline BMI and WC. (5.46 cm vs. 1.83 cm; p=.001)

Conclusions: DOR is significantly associated with gains in WC but not BMI over 5 years of follow-up. Declines in ovarian function preceding menopause are associated with changes in central adiposity.

T-202

Estrogen Levels Modulate Urothelial Barriers and Course of Urinary Tract Infection. Caihong Wang, Jane Symington, Jacob French, Emily Ma, Mallika Anand, Megan Schmid, Lewis Wall, Indira Mysorekar. *Obstetrics & Gynecology, Washington University School of Medicine, St. Louis, MO, USA.*

Urinary tract infections (UTIs), primarily caused by uropathogenic Escherichia coli (UPEC) annually affect over 150 million patients worldwide. A murine model of UTI which phenocopies human infection, has demonstrated UPEC colonize the urinary tract epithelium (urothelium) and persist by establishing quiescent intracellular reservoirs (QIRs) that may serve as reservoirs for recurrent UTIs. Menopausal women are disproportionally affected by recurrent UTIs, yet little is known about the mechanisms underlying this increased susceptibility. We hypothesize that estrogen plays an important role in the maintenance of protective mucosal barriers including glycosaminoglycan layers (GAG) and subsequent recovery post UPEC infection and that hormone therapy with 17 β-estradiol (HT) will positively regulate these processes. We compared the course of UTI in two cohorts of adult mice: one that underwent oopherectomy resulting in surgical menopause (OVX), and one that underwent sham surgery (SHAM). A subset of OVX mice were given HT. All cohorts were then inoculated with a clinical UPEC isolate. Urine was collected daily to determine bacteriuria and bladder tissues were collected upon sacrifice for histological analysis; measuring the GAG layer, and establishment of QIRs. We found that estrogen-deficiency altered thickness of the GAG layers and expression of enzymes involved in GAG biosynthesis; induced a more robust inflammatory response and delayed urothelial regeneration with a remarkably thickened basal cell layer, where urothelial stem cells are located. Excitingly, OVX mice had significantly high bacteriuria and harbored significantly higher numbers of QIRs compared to SHAM mice. HT significantly reduces QIR number, and restored the inflammation levels to SHAM levels. Thus, our findings indicate that loss of estrogen is involved with factors associated with recurrent UTIs such as altered barrier function, delayed regeneration of barriers and increased establishment of QIRs. In addition, HT appeared to decrease the number of QIRs and thus the susceptibility to recurrent UTIs. We plan to compare and validate our experimental findings in post-menopausal women and to establish a link between basic science and clinical practice to better address the clinical burden of UTIs in post-menopausal women.

T-203

Oestrogen Receptor Agonist Regulation of Vascular Tone Is Modulated by Ageing. CJ Nicholson, SC Robson, MJ Taggart. *Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom.*

Objective: Cardiovascular diseases (CVDs) affect the heart, blood vessels and circulatory system. In the UK, CVD accounts for >190,000 deaths per year. The incidence of CVD is lower in women than men between the ages of 30-50 years but thereafter increases substantially. This sharp rise occurs at the same time as women go through the menopause, suggesting a link with oestrogen status. The benefits and risks of oestrogen to the cardiovascular system are still in dispute; two randomised clinical trials failed to show that oestrogen alone, or oestrogen plus progesterone, reduced adverse cardiovascular events in older women. Age- and gender-related changes in the structure and function of the vasculature contribute to the risk of CVD. As such, a greater understanding of how the vascular actions of oestrogens are affected by age is required for more effective cardio-protective treatment options. Here, we investigated the effects of oestrogen compounds on contractility of uterine arteries from young and aged mice.

Methods: Small uterine arteries (<300μm) were dissected from naturally ageing female C57/BL mice and mounted on a wire myograph for isometric force measurement. The effect of increasing concentrations (10nM-30μM) of the oestrogen-receptor (ER) specific agonists, PPT (ER-α) or DPN (ER-β), or the naturally occurring 17-β oestradiol, were examined on U46619 pre-constricted arteries from 3 month-old (oestrous cycling) and 24 month-old (post-oestrous cycling) mice. Responses were analysed using 2-way ANOVA.

Results: U46619 dose-dependent constrictions were reduced in 24 month mice compared to 3 month mice (p<0.05), with maximal constrictions (obtained to 1μM of U46619) of 17.4±1.1kPa and 20.9±1.2kPa respectively. However,

endothelium-dependent acetylcholine-induced relaxation was unaffected. 17-β oestradiol, DPN and PPT each resulted in concentration-dependent relaxation of preconstricted arteries from 3 month old mice. Maximum responses obtained were 57.4±3.5% for 17-β oestradiol, 76.3±6.9% for PPT and 39.1±2.5% for DPN. While PPT and 17-β oestradiol induced similar vasorelaxant effects in 24 month old mice, DPN was without effect (3.5±3.2%).
Conclusions: Reduction of the U46619 constrictive response in older mice suggests an impairment of smooth muscle cell activation with increasing age. The lack of effect of DPN in old mice alludes to age-dependent changes in the ER-specific manner by which oestrogen controls the tone of uterine arteries.

T-204

***S*-nitrosylation of Cofilin-1 Mediates Estrogen Stimulation of Endothelial Cytoskeleton Remodeling and Migration.** Hong-hai Zhang,[1] Seiro Satohisa,[1] Ying-ying Yang,[2] Stephanie Hachey,[1] Lan Huang,[2] Dong-bao Chen.[1] *[1]Dept of Ob/Gyn, Univ of CA, Irvine, CA, USA; [2]Physiol & Biophy, Univ of CA, Irvine, CA, USA.*
Introduction: Estrogens protect the vasculature via stimulating endothelial nitric oxide (NO) production. Adduction of NO moiety to cysteines termed S-nitrosylation (SNO) is an emerging pathway crucial for NO to exert its cellular function. We have recently identified a highly estrogen-induced endothelial *nitroso*-protein cofilin-1 (CFL1). CFL1 is a small actin-binding protein essential for actin remodeling via severing/depolymerizing filamentous *F*-actin. Phosphorylation (pSer3)/de-pSer3 regulate CFL1 activity. However, it is unknown if SNO regulates CFL1 function, thereby mediating estrogen stimulation of endothelial actin remodeling and cell migration. **Objectives**: to determine the functional sequelae of SNO of CFL1 in actin dynamics and cytoskeleton remodeling and cell migration in response to estrogens. **Methods**: Human umbilical cord endothelial cells were treated with estradiol-17β (E2) and SNO and pSer3 CFL1 were determined by biotin switch and immunoblotting. SNO-mimetic or SNO-deficient CFL1 mutants were prepared by site-directed mutagenesis to replace Cys with Ala or Ser, respectively. Recombinant proteins of wild-type (wt)-CFL1 and its mutants were overexpressed in 293T cells and purified; their actin severing activity was determined by real-time fluorescence imaging analysis of *in vitro* formed fluorescently labeled F-actin. The wt-CFL1 and its mutants were transfected into HUVEC for determining the effects of SNO of CFL1 on estrogen-induced filopodia formation and cell migration. **Results**: E2 (10 nM) rapidly stimulated SNO and de-Pser3 of CFL1. wt-CFL1 depolymerized F-actin *in vitro*; its activity was enhanced by Ala but not Ser replacement of C80, which is a major SNO site in CFL1. When overexpressed in HUVEC, basal pSer3 was greatly decreased in Cys80A/S CFL1 mutants. Overexpression of C80A, but not C80S, CFL1 mutant decreased the level of basal *F*-actin and filopodia. E2 stimulated filopodia formation and cell migration in HUVEC, which were significantly affected by overexpression of wt-CFL1 and its C80A mutant. **Conclusion**: SNO of CFL1 regulates its actin severing activity, thereby serving as novel mechanism for mediating estrogen-stimulation of endothelial cell cytoskeleton remodeling and migration (Supported by HL70562 & HL98746).

T-205

Lipid Droplet-Associated PAT Family Proteins Participate in the Release of Prostaglandin E2 in Human Amnion Cells. William E Ackerman, IV,[1] Daniel Montenegro,[2] Sun Kwon Kim,[2] Roberto Romero,[2] Chong Jai Kim,[2,3] John M Robinson,[4] Douglas A Kniss.[1,5] *[1]Obstetrics & Gynecology, The Ohio State University, Columbus, OH, USA; [2]Perinatology Research Branch (NICHD/NIH), Wayne State University, Detroit, MI, USA; [3]Pathology, Wayne State University, Detroit, MI, USA; [4]Physiology & Cell Biology, The Ohio State University, Columbus, OH, USA; [5]Biomedical Engineering, The Ohio State University, Columbus, OH, USA.*
In leukocytes, the formation of lipid storage droplets (LD) is an inducible process implicated in the mediation of arachidonic acid (AA) metabolism and eicosanoid production. LDs accumulate with advancing gestation and labor in the placental amnion during human pregnancy; however, it is not known whether these organelles participate in the release of the prostaglandin E2 (PGE2) that accumulates in the intrauterine environment prior to and during term parturition. Here, we examined the expression of three LD-associated PAT family genes (PLIN1, PLIN2, and PLIN3) in fetal membranes collected in the absence (n=15) or presence (n=18) of spontaneous term labor, and found that there was a significant increase in the expression of both PLIN2 (3.3-fold increase, p=0.002) and PLIN3 (2.2-fold increase, p=0.015) mRNAs following parturition (Mann-Whitney U test). In contrast, PLIN1 mRNA expression was low overall, and not significantly affected by labor. Using amnion mesenchymal cells (AMCs) as an in vitro model system for inflammatory PGE2 production, biochemical fractionation and immunofluorescence co-labeling studies suggested LDs were not intimately associated with the enzymes responsible for PGE2 synthesis, either in the absence or presence of cytokine stimulation. However, LDs were closely associated with PLIN2 and PLIN3. Functional studies using lentiviral-based RNA interference demonstrated that disruption of PLIN2 and PLIN3 expression individually resulted in a significant ($p<0.01$, ANOVA) decrease (25-42%) in cytokine-stimulated PGE2 release in the absence or presence of exogenous AA, while combined PLIN2/PLIN3 knockdown resulted in a 66% decrease in induced PGE2 release. Our results suggest that PLIN2 and PLIN3 contribute to AA metabolism and PGE2 biosynthesis within amnion cells.
Research supported by: K08 HD049628, Subcontract WSU07043 under Contract N01-HD-2-3342, and The Ohio State University Perinatal Research and Development Fund

T-206

Cerclage Improves Neonatal Outcomes in Molar Pregnancy and Coexistent Fetus? Eduardo J Aguin, Tina J Aguin, Victor J Aguin, Ray Bahado-Singh. *Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*
Background: Complete hydatiform mole and coexistent viable fetus is very rare. The probability of carrying a coexistent fetus to term is low due to the risks of miscarriage, preterm delivery, hyperthyroidism, preeclampsia, vaginal bleeding and persistent trophoblastic disease. The use of a cervical cerclage in a patient with cervical insufficiency or advanced cervical dilation in presence of this condition has never been reported. The objective is to show a unique case with a good neonatal outcome in a patient who had a cerclage for cervical incompetence.
Case: A 27 year-old, gravida 7, para 0423, presented with vaginal spotting at 23 weeks and 2 days. Her cervix was 4-5 cm dilated with a bulging bag. Current pregnancy complicated with short cervix (14 mm) and history of 4 preterm deliveries. Ultrasound reported a placental mass versus chorioangioma. Amniocentesis was negative for infection and karyotype 46XY. A cerclage was placed using McDonald technique. Patient received betamethasone for fetal lung maturity and was discharged in stable condition. She then presented with vaginal bleeding and PPROM at 25 weeks and 5 days. She went into spontaneous preterm labor and vaginal delivery of a viable female fetus weighting 625 g and APGAR scores of 7 and 8. D&C was performed after delivery, showing grape-like structures. Placental pathology reported complete hydatidiform mole. Today, this girl is a healthy 4 year old.
Conclusion: The dilemma in the management of molar pregnancy with a coexisting viable fetus is whether to follow them expectantly or to terminate the pregnancy. Partial mole with coexisting live fetus has a high chance of fetal malformation and growth restriction because of associated triploidy. Testing the fetal karyotype is therefore essential. Informing parents about the potential risks and poor outcomes in complete mole and coexistent fetus is necessary. In those patients who understand the risks and still desire all potential interventions, such as cerclage for cervical indications, its placement could be considered.

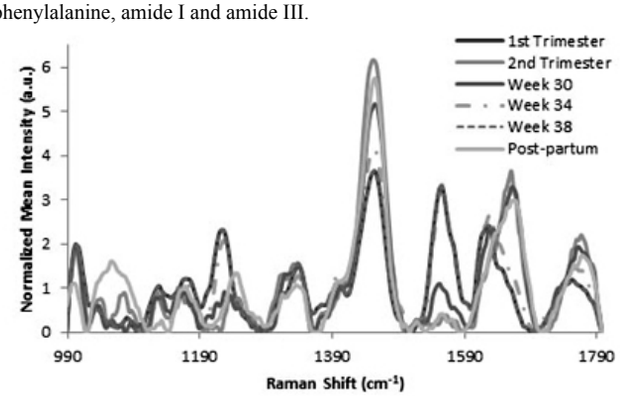
T-207

Steroid Use Reduces Mortality in PPROM at the Borderline of Viability. Ray O Bahado-Singh,[1] Rita Zafra,[1] Devika Maulik,[2] Lindsay Allen,[1] Ismail Mert,[1] Michael Kruger.[1] *[1]School of Medicine, Wayne State University, Detroit, MI, USA; [2]School of Medicine, University of Missouri-Kansas City, Kansas City, MO, USA.*
Objective: To determine whether the use of antenatal corticosteroids in PPROM pregnancies at the borderline of viability, defined as assigned gestational age (GA) 20-24 weeks, improves postnatal mortality.
Study Design: The United States linked birth and infant death data for the years 2004 to 2007 were used. Analysis was limited to reporting states and non-anomalous cases in which the use or non-use of antenatal steroids in PPROM pregnancies at the borderline of viability was reported. The reference group was those at 24^{+0} to 24^{+6} weeks. Logistic regression analysis was used to determine the effect of steroids on postnatal death after adjusting for multiple potential confounders: GA , BW (to control for errors in GA), year of birth, race, parity, delivery route, gender , maternal diabetes, hypertension and eclampsia. The odds of early (ED) neonatal death (1-6 days), (NND) neonatal death (1-27 days) and (ID) infant death (0-365 days) with corticosteroid vs non-use were compared. Chi square, logistic regression adjusted OR (95% CI) and p-values were determined.
Results: There were a total of 5,172 eligible cases of which 1802 (34.8%) died in the first year. Antenatal steroids was used in 3.1%, 4.9%, 9.6% and

17.4% and 25.6% of cases respectively for each week from 20-24 with overall usage of 14.4%. Overall, antenatal steroids significantly reduced rates of ID (25.1% vs 36.5%, p <0.001), NND (20.0% vs 34.8%, p<0.001) and ED (15.8 vs 32.7% ,p<0.001). Adjusted OR (95% CI) for antenatal steroids based on logistic regression were, 0.65 (0.53, 0.80, for NND, 0.74 (0.61, 0.90) for ID , and ED: 0.58 (0.46, 0.72), p<0.001 for all categories.
Conclusion: There is an absence of data on the benefits of steroids in PPROM at the borderline viability. Our data provide evidence of a significant reduction in postnatal deaths with steroid use in this gestational period.

T-208

Detecting Biochemical Changes in the Human Cervix with Raman Spectroscopy. Elizabeth Vargis,[1] Kelly A Bennett,[2] Nathan Webb,[2] Ayman Al-Hendy,[3] Anita Mahadevan-Jansen.[1] *[1]Biomedical Engineering, Vanderbilt University, Nashville, TN, USA; [2]Obstetrics and Gynecology, Vanderbilt University, Nashville, TN, USA; [3]Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*
Objective: Prematurity is the 2nd leading cause of neonatal mortality and can lead to significant neonatal morbidity. Currently, there are few ways to accurately detect preterm cervical changes, impairing efforts to prevent and treat preterm labor (PTL). Our goal is to develop an optical technique to noninvasively monitor cervical ripening and determine which changes correspond to the onset of labor. Raman spectroscopy is an optical method that measures changes in vibrational modes of tissues based on its biochemical composition. Our group has previously shown that cervical tissues yield distinct Raman spectral signatures that can be used to distinguish cancerous from non-cancerous areas with over 99% accuracy. To our knowledge, our study is the first group to use Raman spectroscopy as an optical method for detecting cervical changes associated with pregnancy.
Methods: Spectra were acquired from the cervix of women [n=40 pregnant (P), 20 non-pregnant (NP)] over the course of 1.5 years, using a portable, in vivo, Raman system with a fiber optic probe. The exposure time was 3 seconds of 785nm light at 80 mW power.
Results: Raman spectra from humans show distinct spectral differences during pregnancy, corresponding to differences in cervical DNA, lipid, and protein content (Figure). Significant differences in peak intensity and peak widths were noted at multiple locations, in the measured spectra (Student's t-test, p<0.05), corresponding to phospholipids, C-O stretching (2 peaks), carotenoids, phenylalanine, amide I and amide III.



Conclusion: Raman spectroscopy is a noninvasive test that effectively detects statistically significant biochemical changes that occur in the cervix during pregnancy. These results suggest that Raman spectroscopy could potentially lead to an accurate method of studying how the cervix changes during pregnancy and identify women at risk for PTL.

T-209

Adverse Childhood Experiences Are Associated with Spontaneous Preterm Birth: A Case-Control Study. Inge Christiaens,[1] Kathleen Hegadoren,[2] David M Olson.[3] *[1]Physiology, University of Alberta, Edmonton, AB, Canada; [2]Nursing, University of Alberta; [3]Physiology, Obstetrics & Gynecology, Pediatrics, University of Alberta.*
Introduction Preterm birth is the leading cause of mortality and morbidity in newborn infants. Despite considerable research, the majority of preterm births are still largely unexplained, due to the complexity and number of etiologies. Maternal stress during pregnancy is increasingly recognized as a variable of interest, however its contribution to the risk of spontaneous preterm birth remains controversial. Studies examining the effect of maternal stress during pregnancy on preterm birth show varied results, therefore, examining the exposure to stressors over a mothers' life course might give a better perspective on the contribution of stress to preterm birth.
Objective The aim of this study was to explore the associations between various lifelong stressors and protective factors and spontaneous preterm birth.
Methods This is a retrospective case-control study based in Edmonton, Alberta. Cases (n=75) were mothers with a spontaneous singleton preterm birth (<37 gestational weeks) without preterm premature ruptures of membranes. Controls (n=148) were mothers with an uncomplicated singleton term birth (38-42 gestational weeks) without a history of preterm birth. Sociodemographic and medical data were collected. A telephone questionnaire was administered to assess measures of global perceived stress before and during pregnancy, common stressors, social support (ISEL), life events, coping (COPE), adverse childhood experiences (ACE), abuse (AAS) and depression. A total stress score was also computed. Univariate and multivariate logistic regression analysis were performed.
Results Univariate analyses showed that maternal age, smoking, low education, a high total stress score and a high ACE score (≥2 exposures to adverse childhood experiences) were associated with spontaneous preterm birth (p<0.05). Multiple logistic regression demonstrated that a high ACE score was independently associated with preterm birth (adjusted odds ratio 2.01; 95% confidence interval 1.07-3.79).
Conclusions Exposure to 2 or more adverse childhood experiences is associated with a two-fold increase in the risk of spontaneous preterm birth. This is the first evidence showing a relationship between adverse childhood experiences and preterm birth.
Acknowledgement Supported by AIHS ITG Preterm Birth and Healthy Outcomes Team (PreHOT) award.

T-210

Human Placental and Myometrial Arteries Differ in Their Ca^{2+}-Sensitivities of Force Production. AC Dordea, M Sweeney, SC Robson, MJ Taggart. *Institute of Cellular Medicine, Newcastle University, United Kingdom.*
Objective: During gestation, the placental circulation develops *de novo*, whilst the uterine vasculature undergoes dynamic remodelling to facilitate provision of oxygen and nutrients from the mother to the placenta and fetus. Such distinct features of these circulations suggest that regulation of vascular function may differ. Although contraction of vascular smooth muscle is determined by the relative activities of Ca^{2+}-calmodulin-dependent myosin light chain kinase and myosin light chain phosphatase, there is surprisingly little information on the nature of Ca^{2+}-sensitive force production in human placental (PA) or myometrial arteries (MA). Therefore, this study aimed to compare between human PA and MA (i) the Ca^{2+}-dependent sensitivity of force production and (ii) the enhancement of Ca^{2+}-dependent contractility conferred by physiologically important G-protein-coupled agonist activation.
Methods: PA and MA were dissected from biopsies obtained from pregnant women at term following written informed consent. Arteries were mounted for isometric tension measurement, contractility tested and subsequently permeabilised with α-toxin to enable experimental control of $[Ca^{2+}]$ surrounding the myofilaments.
Results: Intact PA constricted significantly less to high K^+ (KPSS) than MA (5.7±0.1 kPa, n=17 vs 8.8±0.2 kPa, n=15, p<0.05) yet in permeabilised tissues the magnitude of Ca^{2+}-induced dose-dependent constrictions (pCa9-pCa4.5 in 9 incremental steps) were similar (2-way ANOVA). However, the sensitivity of constriction to Ca^{2+} was significantly lower in PA than MA ($logEC_{50(PA)}$=-6.26±0.05 (n=9) and $log_{EC50(MA)}$=-6.64±0.07 (n=8), p<0.05, unpaired student *t-test*). The thromboxane agonist mimetic U46619 induced significant enhancement of force accompanied by a significant sensitisation to each activating $[Ca^{2+}]$ relative to time control (TC) in PA ($log_{EC50(TC)}$=-6.26±0.05 and $logEC_{50(U46619)}$=-6.97±0.06, p<0.05) and MA ($logEC_{50(TC)}$=-6.64±0.07 and $logEC_{50(U46619)}$=-7.05±0.05, p<0.05). The extent of the U46619-induced sensitisation to Ca^{2+} was thus greater in PA in order to match that in MA.
Conclusion: These data indicate that the contractile capacities of intact human placental arteries (to KPSS), and the Ca^{2+}-sensitivity of force production in permeabilised vessels, are less than that of myometrial arteries. This informs our increasing awareness of how the molecular mechanisms of tone regulation may differ in the human placental and uterine circulations.

T-211

Chronic Stress Does Not Decrease Leukocyte Chemotaxis of Uterus and Cervix and Is Not Related to Maternal Systemic Progesterone Levels in Rats. Nardhy Gomez-Lopez,[1] Jerrah Robbins,[2] Erin Falkenberg,[2] David M Olson,[1] Gerlinde Metz.[1] *[1]OB/GYN, Pediatrics & Physiology, University of Alberta, Edmonton, AB, Canada; [2]Canadian Centre for Behavioural Neurosciences, University of Lethbridge, Lethbridge, AB, Canada.*

Objective: Normal labor is accompanied by infiltration of leukocytes into intrauterine tissues. This phenomenon is regulated by production of chemotactic factors by these tissues and by the sensitivity of circulating leukocytes to these factors. Previously, we demonstrated that both leukocytes and tissues have maximal leukocyte chemotaxis at term labor and that mild chronic stress during late pregnancy alters maternal and offspring behaviours and the timing of delivery in rats. Given the close association between stress and the immune system, we hypothesized that chronic stress during late pregnancy alters leukocyte chemotaxis.

Methods: Pregnant Long-Evans dams were stressed from day 12 to 18 of gestation with 20 min of restraint randomly alternated with a 5-min swim. Gestation lengths were monitored with infrared cameras. Uterus and cervix from stressed dams were taken at gestational day (GD) 22 during early labor (n=8) and from unstressed dams at GD22 without labor (n=5; normal labor occurs at GD22.5). Tissue extracts were prepared and maternal peripheral leukocytes were collected from unstressed dams at 22GD, and isolated using a Ficoll gradient. Chemotaxis assays were performed using a validated Boyden chamber. Leukocyte numbers were determined using flow cytometry. Additionally, progesterone systemic levels were measured in unstressed and stressed rats at GD19, 20 and 22. Statistics: Mann-Whitney U test, significance $p<0.05$.

Results: Leukocyte chemotaxis of uterus and cervix were greater in unstressed dams than in stressed dams (attracted leukocytes in 30 sec: uterus 3556 ± 484 vs. 112 ± 23, $p= 0.002$ and cervix 3486 ± 1080 vs. 105 ± 12, $p = 0.035$). As expected, systemic progesterone concentrations decreased following luteolysis, and were not different in stressed and unstressed dams at any time-point.

Conclusion: We confirmed our hypothesis that leukocyte chemotaxis of uterine and cervical tissues in stressed dams is altered and this was unrelated to maternal serum progesterone concentrations. These data suggest that physiological processes in stressed rats may be fundamentally changed.

Supported by AIHS PreHOT, CIHR, MTPRF.

T-212

Chemoattractant Activity of Cervix and Ovary Is Not Increased at Term or RU486-Induced Preterm Labor in the Guinea Pig. N Gomez-Lopez,[1] WC Tong,[2] S Tanaka,[1] DM Olson,[1] GN Europe-Finner,[2] MJ Taggart,[2] BF Mitchell.[1] *[1]OBS/GYN, University of Alberta; [2]ICM, Newcastle University.*

Objective: Normal labor is accompanied by infiltration of leukocytes into reproductive tissues. Current understanding is that these leukocytes participate in cervical repining and the ovarian processes of ovulation and luteolysis. Leukocyte infiltration is likely regulated by production of chemoattractant activity (CA) of factors produced in these target tissues. Using a guinea pig model of normal term and RU486-induced preterm parturition, we measured CA of extracts of cervix and ovary on a pool of leukocytes obtained from term gestation (>d64, normal gestation is 68 ± 0.3 d). We hypothesized that the leukocyte CA would increase through late gestation and during spontaneous or induced labor.

Methods: Cervix and ovary were isolated from guinea pigs at late gestation (LG; d50-64; n=11), term gestation not-in-labor (TNIL; > d64; n=5) and term gestation in spontaneous labor (TSL, >d64, n=6). Preterm labor (PTL, n=6) was induced using the progesterone (P4) antagonist RU486 (3 mg/kg body weight) injected on d55 and 56. Birth occurred at 47 ± 7h after the first injection. Controls (CPTL; d51-59; n=5) received vehicle only. Tissue extracts were prepared. Maternal peripheral leukocytes were isolated using a Ficoll gradient. CA was measured using a validated Boyden chamber assay. Leukocyte numbers and subpopulations were determined using flow cytometry. Statistical analyses used Kruskal-Wallis and Mann-Whitney tests with significance at $P<.05$.

Results: CA of cervical extracts did not change throughout late gestation or during term spontaneous labor. However, CA for granulocytes was significantly lower in RU-induced PTL animals than in CPTL and TSL animals. CA of ovarian extracts for granulocytes and lymphocytes peaked in the TNIL animals and was significantly decreased in TSL animals. In addition, CA of ovarian extracts was significantly lower for lymphocytes in PTL compared to CPTL and TSL animals.

Conclusion: CA in cervical extracts does not change significantly through late pregnancy and parturition in the guinea pig. However, cervical CA is significantly decreased following RU486-induced PTL. Although, CA of ovarian tissue decreases in TSL, it decreases even more in RU486-induced PTL. These data support the concept that cervical and ovarian CA is partially dependent on P4 and is not required for parturition.

Funding: MRC in UK; AIHS and WCHRI in Alberta

T-213

Oxytocin Mediates COX-2 Expression Via NF-κB and MAPK Pathway. SH Kim,[1] A Blanks,[2] S Thornton,[2] M Johnson,[1,3] PR Bennett,[1] V Terzidou.[1,3] *[1]Parturition Research Group, IRDB, Imperial College, London, United Kingdom; [2]Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom; [3]Obstetrics and Gynaecology, Chelsea & Westminster Hospital, London, United Kingdom.*

Background: Human labour is associated with an increase in prostaglandin (PG) and inflammatory cytokine synthesis within the fetal membranes. Incubation of pre-labour amnion cells with oxytocin (OT) results in a marked increase in PGE2 synthesis and upregulation of COX-2. The aim of this study was to identify the regulatory pathways involved OT-mediated COX-2 expression in human amnion and to compare this with the effect of IL-1β.

Methods: Primary amnion cell cultures were established from patients having elective caesarean section at term, before labor onset (L-). Cells were treated with Oxytocin (OT, 10-7M) or IL-1β (1ng/ml). Nuclear-cytosolic and whole cell proteins were extracted for Western blot analysis. Equal proteins loadings were verified using a β-actin control. Amnion epithelial cell transfections were carried out using Amaxa nucleofector kit (Lonza).

Results: siRNA mediated knockdown of p65 NF-κB subunit resulted in suppression of both IL-1β and OT induced COX-2 upregulation (n=3 patients; $p<0.05$, ANOVA). We found OT treatment increases nuclear translocation only of p65 whilst IL-1β significantly increases nuclear translocation of p65, p50 and RelB subunit. Both OT and IL-1β activated p65 and IKKα/β, and resulted in degradation of IκBα. Pre-treatment with IKKβ inhibitor (TPCA-1) significantly decreased the upregulation of COX-2 by OT (n=3 patients; $p<0.05$, ANOVA). We found that both OT and IL-1β activate ERK1/2 and p38 kinase, but only IL-1β activates JNK. Inhibition of ERK1/2 and p38 resulted in suppression of COX-2 upregulation by OT or IL-1β, and of OT, but not IL1b, stimulated activation of NF-κB pathway (n=3 patients; $p<0.05$, ANOVA). Pre-labour amnion epithelial cells treated with OT showed significant increases in mRNA expression of various NF-κB-regulated genes, including IL-8, CCL2, CCL5 and COX-2 (n=3 patients, duplicate; $p<0.05$, ANOVA).

Conclusion: We demonstrate that OT activates NFkappaB in human amnion by a novel mechanism, which, unlike IL1b, requires both IKK and MAPK activation. Our findings suggest a potential role of the OTR-antagonist and NF-κB inhibitors in the management of activation of human amnion and the onset of labour.

T-214

Gene Networks and Cellular Functions That Are Regulated through Progesterone and Glucocorticoid Receptors in Human Myometrial Cells. Kaiyu Lei,[1] Suren R Sooranna,[2] Phillip R Bennett,[1] Mark R Johnson.[2] *[1]Surgery & Cancer, Imperial College, IRDB, London, United Kingdom; [2]Academic Obstertrics & Gynaecology, Imperial College, Chelsea & Westminster Hospital, London, United Kingdom.*

Introduction: Inflammation plays a central role in many human diseases. Several nuclear receptors have been found to be key regulators in response to inflammatory processes. Human parturition resembles an inflammatory reaction, where progesterone (P4) and progesterone receptors (PRs) have already been demonstrated to suppress contraction-associated gene expression. In our previous studies, we have found that the actions of progesterone, including progesterone-induced gene expression and progesterone's anti-inflammatory effect, are mediated by both PR and GR. Consequently, the gene networks and cellular functions regulated by P4 or MPA in the presence or absence of PR and GR in human myometrial cells were investigated by microarray analysis with the aim of improving our understanding of the role of PR and GR in human parturition.

Methods: Human myometrial cells were were transiently transfected with siRNA and after 4 days were exposed to IL-1β (5ng/mL), MPA (1 uM) and P4 (10 uM), either alone or in combination for 6 h, and then total RNA and protein were extracted. The effectiveness and selectiveness of PR and GR knockdown were assessed by western blots. 3 representative samples with different endogenous PR levels, but similar GR levels, were chosen for Affymetrix Human Genome U133 plus 2.0 Array. Independent studies by qPCR were performed on candidate genes in order to confirm the results from cDNA microarray analysis.

Results: 8 gene lists were generated by combining the top 50 up-regulated and the top 50 down-regulated genes from each comparison. In each list, genes with $p < 0.05$ were analyzed. 8 genes of interest were confirmed to be regulated by P4. Of these, HSD11β1 was solely mediated by PR, and 4 genes (ERRFI1, DUSP1, COX-2 and TNFRSF11B) were GR dependent. FKBP5 and IL-1β were shown to be mediated by both steroid receptors. MPA not only regulated most of the P4-responsive genes, but also other genes that were driven by dexamethasone.
Conclusions: This study has shown that MPA and P4 regulate distinct gene networks and cellular functions due to, at least in part, the glucocorticoid effect of MPA, with only a few genes being sensitive to both MPA and P4. This study also suggests a more prominent role of GR in regulating the myometrial functions during human parturition.

T-215

MKP-1 and GR Mediate Progesterone-Induced COX-2 Repression in Human Myometrium. Kaiyu Lei,[1] Suren R Sooranna,[2] Phillip R Bennett,[1] Mark R Johnson.[2] *[1]Surgery & Cancer, Imerial College, IRDB, London, United Kingdom; [2]Academic Obstetrics & Gynaecology, Imerial College, Chelsea & Westminster Hospital, London, United Kingdom.*
Introduction: Progesterone plays anti-inflammatory roles to maintain uterine quiescence during pregnancy and its functional withdrawal leads to the onset of labour. Prostaglandins play a central role in human parturition by inducing cervical remodelling and uterine contractility. COX-2 controls the rate-limiting step of pro-inflammatory metabolite biosynthesis. Progesterone receptor (PR) mediates its anti-inflammatory actions by inhibiting the activity of NF-κB. However, glucocorticoid receptor (GR) appears to be a key mediator of progesterone actions in human myometrium. In this study, we investigate the mechanism of GR-mediated repression of IL-1β-induced COX-2 expression.
Methods: Human myometrial cells were cultured to 90% confluency, serum starved overnight and pre-incubated with 0.5μM Trichostatin A (TSA, an HDAC inhibitor) for 1h or 0.5μM sanguinarine (Sang, a MKP-1 inhibitor) for 30 min before being exposed to IL-1β (5ng/mL), MPA (1uM) and P4 (10uM), either alone or in combination for 6 h. Cells were also transiently transfected with siRNA. At the end of incubations, RNA or protein were analyzed by qPCR and western blotting (n=6). For CHIP assays, cells were treated with 1% formaldehyde, sonicated and immunoprecipitated using anti-p65 antibody. DNA was isolated and two NF-κB binding sites were measured by qPCR (n=3).
Results: MPA/P4 did not alter p65 binding to the proximal and distal NF-κB binding elements of the COX-2 promoter, but GR knockdown enhanced p65 binding to both elements and this was not associated with an increase in COX-2 mRNA expression. The HDAC inhibitor, TSA, did not change the MPA inhibition of IL-1β-driven COX-2 expression and but tended to enhance the effect of P4. GRIP-1 knockdown did not alter the MPA/P4 inhibition of IL-1β-driven COX-2 expression. However, when the MKP-1 activity was inhibited, the ability of MPA/P4 to inhibit IL-1β activity was significantly reduced. Both P4 and MPA increased MKP-1 expression, which was mediated by GR.
Conclusions: These data suggest that progesterone acts via progesterone-induced and GR-mediated MKP-1 activation to repress IL-1β-driven COX-2 expression, whereas the phosphorylation, nuclear translocation and DNA binding activity of p65 as well as the HDAC activation and the recruitment of GRIP-1 on the GR-p65 protein complex appear to be not involved.

T-216

Androgen and Estrogen Receptor Expression Is Differentially Regulated in Fetal Programmed Kidneys: Possible Mechanism for Gender-Based Reduced Nephrogenesis and Hypertension. Thomas R Magee,[1] Cynthia C Nast,[2] Sanaz A Tafti,[1] Thuy D Nguyen,[1] Eric L Lam,[1] Kevin A Amaya,[1] Michael G Ross,[1] Mina Desai.[1] *[1]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Pathology, Cedars-Sinai Med. Ctr, Los Angeles, CA, USA.*
Objective: Human and animal intrauterine growth restriction (IUGR) results in reduced nephron number, adult hypertension, and possible renal disease. Males are more affected than females, suggesting a sex hormone-related mechanism, whereby testosterone may increase and estrogen may reduce susceptibility to effects of IUGR. Our model of rat IUGR shows alteration in nephrogenic gene expression, reduced nephron number, and hypertension in male offspring. Additionally, microarray data detected a large increase in androgen receptor mRNA expression in IUGR fetal kidneys (Reprod Sci. 2011 18(6):563). We hypothesized that gender-based IUGR programmed nephrogenesis is mediated by sex hormones. We measured expression of androgen (AR) and estrogen (ER) receptors in male and female (embryonic day 20) kidneys. Additionally we examined whether a down-stream gene, KAP (Kidney Androgen Regulated Protein), known to be under the control of androgen receptor was affected by IUGR.
Study Design: Pregnant rat dams were fed either ad libitum diet (control) or were 50% food restricted (IUGR) from E10. At E20, male and female offspring kidneys (n=6 dams for MUN and control) were examined for mRNA expression by SYBR green relative qPCR and protein expression by immunohistochemistry. Values are expressed as fold change.
Results: AR mRNA was increased in male (2.7 fold, p=0.04) but decreased in female (0.33 fold, p=0.01) IUGR kidneys. AR protein was increased in male IUGR kidneys. In male IUGR kidney, ERα mRNA was unchanged though ERβ mRNA was down-regulated (0.26 fold, p=0.03). In females, both ERα and ERβ were upregulated (1.4 and 2.7 fold, respectively; $p<0.05$). KAP mRNA, known to be transcriptionally induced by AR, was increased in male IUGR kidneys (2.8 fold, p=0.04) but decreased in females (0.29 fold, p=0.04).
Conclusions: Maternal undernutrition-induced IUGR increases expression of AR in males while decreasing expression in females. Conversely, ERα and ERβ were unaffected or down-regulated in male IUGR but increased in female IUGR kidneys. These data suggest that both AR and ER expression may play a role in mediating the increased severity of IUGR in males.

T-217

Immunomodulation in Preterm Labour: An Answer to *In Utero* Neurological Insult. Johann Malawana,[1] Laura Howe,[2] Renyi Hua,[2] Bronwen Herbert,[1] Suren R Sooranna,[1] Phillip R Bennett,[2] Mark R Johnson.[1] *[1]Academic Obstetrics & Gynaecology, Imperial College, Chelsea and Westminster Hospital, London, United Kingdom; [2]Surgery & Cancer, Imperial College, IRDB, London, United Kingdom.*
Introduction:
Rates of preterm delivery are increasing across the developed world and range between 8-13%. Overall, preterm delivery is the most important cause of perinatal morbidity and mortality; with most problems occurring in babies born before 32 weeks. In this group, preterm labour (PTL) is most commonly caused by infection/inflammation, prompting the search for agents that can modulate the maternal inflammatory response, thereby reducing rates of PTL. We use a proven mouse model of inflammatory PTL based on the administration of lipopolysaccharide (LPS), a major cell-wall component of gram-negative bacteria, which activates the innate immune system via the toll-like receptor type 4. In this study, we used rolipram and YM976, both phospodiesterase (PDE) 4 inhibitors to modulate the maternal immune system in an attempt to improve rates of pup survival and prolong pregnancy. YM976 is 1000 times more potent that Rolipram so allowed us to compare the effect with potency of PDE4 inhibition.
Methods:
10ug of LPS was administered into the right horn of the uterus, at laparotomy, performed on E16 of gestation in CD1 outbred mice. 2 hours prior to this we administered either 5mg/kg of YM976, 2mg/kg of rolipram or DMSO vehicle diluted in PBS (control) via intraperitoneal injection.
Results:
The rolipram group of mice showed a significant delay in delivery time compared with the control group, with a p value of 0.0253. However, pup survival was not significantly improved. The YM976 group did not show significant improvement in delay of labour p=0.0923, although this may have been improved with an increase in numbers of mice used. However, due to significantly higher likelihood of welfare problems, continuing experiments with YM976 were abandoned.
Conclusions:
These data suggest that inhibition of PDE4 may prolong pregnancy, but that it does not appear to improve fetal outcomes. Further studies will define the mechanisms by which rolipram delays parturition and define whether this approach might be clinically useful in the future.

T-218

Elucidating Mechanisms of Fetal Injury from Intrauterine Inflammation. Monique E Maubert, Amy G Brown, Michal A Elovitz. *Maternal and Child Health Research Program, Department of Obstetrics and Gynecology, CRRWH, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.*
Introduction: Intrauterine infection and inflammation are an established risk factor for preterm birth, as well as adverse neurobehavioral outcomes for offspring. In an established mouse model, we have demonstrated that intrauterine inflammation results in fetal neuronal injury. Whether endotoxin

in the uterine cavity can reach the feto-placental unit and have a direct effect on the fetus and fetal brain is not known. This study sought to determine the trafficking patterns of endotoxin from the uterine cavity to the feto-placetal unit.
Methods: Using a mouse model, on E15 of gestation, CD1 dams received intrauterine infusion of: 1) Alexa-Fluor 488-LPS at 250ug/dam (Fluor250), 2) 50ug/dam (Fluor50), or 3) saline at 100ul/dam (NS). Fetal brains (FB), placentas (PL), uteri (UT), maternal spleens (MSpl), maternal serum (MS), and amniotic fluid (AF) were harvested at 6 hours post-infusion. FB, PL, UT, and MSpl were immediately fixed in 4% paraformaldehyde. Tissues were then paraffin-embedded and sectioned. Slides were stained with Texas-Red Phalloidin and imaged on a Zeiss wide field microscope at 20X, 40X and 100X. MS and AF were collected, frozen in liquid nitrogen and stored at -80C for use in Limulus Amebocyte Lysate (LAL) Assay.
Results: Photos of punctate green fluorescence (indicative of the Alexa-Fluor 488-LPS) were captured in both the UT and the fetal side of the PL of Fluor250 dams; however, we did not capture similar punctate green fluorescence in the Fluor50 dams, nor in the FB nor the MSpl of either Fluor cohort. LAL Assay yielded positive endotoxin presence in the AF of Fluor250-treated dams; as expected, there was no presence of endotoxin in the NS-treated dams. There was also no presence of endotoxin in the MS of all treatment groups.
Conclusions: Endotoxin that is present in the uterine cavity is able to reach the feto-placental unit in the absence of hematogenous spread in the mother. As endotoxin can reach the placenta, it is likely that pathogen by-products can activate an immune response in the placenta that may lead to fetal injury; these biological events may occur independent of an immune response in the uterus or systemically in the mother. Understanding the trafficking of pathogens and their by-products in infection-induced fetal injury can lead to improved therapeutic strategies to reduce adverse outcomes.

T-219

A Maternal Immune Response Is Necessary for Fetal Injury from Intrauterine Inflammation. Monique E Maubert, Amy G Brown, Michal A Elovitz. *Maternal and Child Health Research Program, Department of Obstetrics and Gynecology, CRRWH, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.*
Introduction: Intrauterine inflammation is an established risk factor for fetal brain injury and adverse neurobehavioral outcomes for exposed offspring. We previously demonstrated that maternal toll-like receptor-4 (TLR4) is necessary for inflammation-induced preterm birth. Current studies were performed to determine if maternal TLR4 is necessary for fetal brain injury or whether TLR4 activation in the feto-placental unit is sufficient to cause fetal harm.
Methods: TLR4 mutant mice (HEJ) and wild-type mice (WT, CD1) were used for these studies. Three dam cohorts were used: 1) WT embryos transferred into pseudopregnant WT dams (WT/WT); 2) WT embryos transferred into pseudopregnant HEJ dams (HEJ/WT); and 3) naturally conceived WT dams (WTnat). All dams received intrauterine infusion of LPS at 250ug (LPS250) or saline (NS). Fetal brains (FB), placentas (PL), uteri (UT), maternal serum (MS), and amniotic fluid (AF) were harvested at 6 hours post-infusion. RNA from FB, PL and UT were isolated and cytokine expression was assessed by qPCR. IL-6 expression was assessed in MS and AF by ELISA.
Results: IL-6 levels were not detectable in MS from any cohort. IL-6 was significantly increased in the AF of WTnat LPS250 versus NS ($p < 0.01$), but levels were not different in the AF of HEJ/WT. IL-1B, IL-6 and TNF-a mRNA expression was assessed in WTnat, HEJ/WT and WT/WT cohorts (Table 1). WT/WT served as a procedural control cohort and demonstrated similar cytokine expression increases as WTnat.

Table 1: Fold change in cytokine mRNA in PL, UT and FB of WTnat and HEJ/WT cohorts.

	WTnat PL	HEJ/WT PL	WTnat UT	HEJ/WT UT	WTnat FB	HEJ/WT FB
IL-1B	12.4 ($p < 0.01$)	1.7 (ns)	16.4 ($p < 0.01$)	3.3 ($p = 0.03$)	6.5 ($p = 0.04$)	0.8 (ns)
IL-6	14.0 ($p < 0.01$)	2.2 ($p = 0.04$)	102.5 ($p = 0.03$)	3.4 (ns)	3.1 ($p = 0.04$)	0.5 (ns)
TNF-a	19.5 ($p < 0.01$)	2.2 (ns)	44.3 ($p = 0.03$)	4.0 ($p = 0.02$)	9.9 ($p < 0.05$)	0.6 (ns)

Fold Change is mean expression in LPS-exposed by expression NS-exposed tissues; ns = not significant.

Conclusions: In the absence of maternal TLR4, endotoxin in the uterine cavity fails to induce an immune response in the uterus. In the absence of this maternal immune response, there is no placental immune response or notable cytokine response in amniotic fluid or fetal brain. Acute brain injury (6 hours) from intrauterine inflammation is dependent on a local maternal immune response from TLR4 activation. Targeting TLR4 activation in the mother may be a potential therapy to prevent inflammation-induced fetal injury.

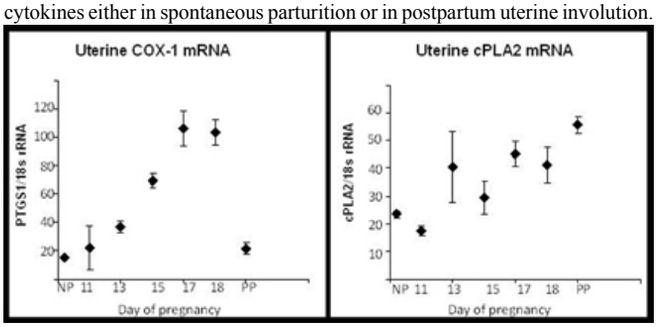
T-220

Histone Deacetylases and Immune Response in the Placenta. Sheryl K Munro,[1,2] Murray D Mitchell,[1,2,3] Anna P Ponnampalam.[1,2] *[1]The Liggins Institute, The University of Auckland, Auckland, New Zealand; [2]The National Research Centre for Growth and Development, The University of Auckland, New Zealand; [3]Centre for Clinical Research, University of Queensland, Brisbane, Australia.*
Introduction: Cytokines play critical roles in parturition, cervical dilation, rupture of membranes and uterine contractility by inducing the production of prostaglandins and matrix metalloproteinases. Cytokines are also produced in response to infection - a major causative factor in the preterm initiation of labor. The precise mechanism of cytokine regulation in gestational tissues (GT) is not well understood. We have previously identified tissue specific differences in cytokine production by GT in responses to LPS and the histone deacetylase (HDAC) inhibitor trichostatin A (TSA).
Objectives: To determine whether differences in response to TSA treatment reflect underlying differences in HDAC expression and to determine the changes in the expression and regulation of HDACs in response to LPS and TSA in placental villous explants.
Methods: Gene expression of HDACs was quantified by real-time PCR in pre-labour term GT. Villous explants were isolated from term placentae and treated with LPS and/or TSA. Explants and media were then collected (0-12hrs (2 hourly), 18, 24, 48hrs) and a time course for HDAC and cytokine mRNA expression has been established using real-time PCR.
Results: All 18 HDACs showed distinct tissue specific expression profiles. Notably in contrast to other HDACs, expression of HDAC4, which associates with HIF1α, was highest in the chorio-decidua (>5 fold compared to villous, $p<0.05$; >2 fold compared to amnion, $p< 0.01$). Data from explant studies show differential cytokine responses to TSA treatment, with TSA co-treatment increasing the expression of IL1β in response to LPS, while mitigating the LPS induced increase in TNFα expression. Interestingly, TSA which inhibits HDAC deacetylase activity also appears to transiently feedback on HDAC expression with increased HDAC1 and HDAC3 expression observed concurrently with the peak co-treatment effects on cytokine expression.
Conclusion: Our data suggest a role for specific HDACs in cytokine regulation in GT and may indicate the involvement of specific non-histone binding partners rather than a general histone hyperacetylation. These studies may help elucidate the role of HDACs in cytokine regulation and lead to an understanding of how to manage and prevent the deregulation of the inflammatory response in the placenta.

T-221

Real-Time PCR Quantitation of Select Biochemical Markers in Mouse Uterus over the Course of Gestation: Implications for Function. Joshua Reese,[1] Vladimir Ilievski,[1] Xiaowu Qu,[1] Emmet Hirsch.[1,2] *[1]Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, IL, USA; [2]Obstetrics and Gynecology, Pritzker School of Medicine, University of Chicago, Chicago, IL.*
Objective: To quantify uterine mRNA concentrations of select biochemical markers at discrete time points over the course of normal gestation in mice.
Methods: Total RNA was extracted from the uteri of non pregnant and healthy pregnant CD-1 mice at gestational days 11, 13, 17, 18, and postpartum. Real-time PCR using ABI TaqMan reagents normalized to the expression of 18s rRNA was conducted for cytosolic phospholipase A2 (cPLA2), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), prostaglandin-endoperoxide synthase 1 (COX-1), COX-2, prostaglandin F receptor (PTGFR), inducible nitric oxide synthase (NOS II), endothelial nitric oxide synthase (NOS III), transforming growth factor beta receptor 2 (TGFβR2), and tumor necrosis factor (TNF). Three to seven mice were studied at each time-point. Parametric and non-parametric tests were used to determine whether intergroup differences were significant. Trend ANOVA was used to assess trends over gestation and weighted P values are reported. $P < 0.05$ was considered significant.
Results: All deliveries occurred at night. Compared with the day prior to delivery, uterine IL-1β (P=0.033), COX-2 (P=0.034), and TNF (P=0.006) mRNA concentrations were significantly increased immediately postpartum, while COX-1 ($p<0.001$) was decreased. cPLA2 and COX-1 mRNA concentrations trended upward over the gestational period ($P<0.001$ for both). No significant changes over gestation were observed for IL-6, NOS II, NOS III, and TGFβR2.
Conclusions: Enzymes important for the production of prostaglandins and their precursors (cPLA2 and COX-1) increase in mouse uterus over the course of gestation, suggesting the presence of a priming process for prostaglandin

activity as parturition nears. The pro-inflammatory cytokines IL-1β and TNF are increased in the immediate post-partum uterus, suggesting a role for these cytokines either in spontaneous parturition or in postpartum uterine involution.



T-222

Effects of Parenteral and Intrauterine Progesterone (P4) on Uterine EMG Activity, Delivery and Cervical Ripening during Pregnancy. Shao-Qing Shi, Leili Shi, Bhargavi Pulluri, Robert E Garfield. *Ob/Gyn, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA.*

How P4 acts to inhibit birth is unclear. **Objective:** To evaluate if P4, given by subcutaneous (s.c.) injection or by direct intrauterine infusion can affect uterine electromyographic (EMG) activity and cervical function during term delivery. **Study Design:** Timed-pregnant Sprague-Dawley rats (n = 46, normal delivery on gestation day 22) were used. Some rats were treated s.c. daily 1 or 2 days (i.e. days 20 or 21) with vehicle (controls, CTL) or P4 (4 mg, crystalline in ethanol:oil or soluble P4 equivalent to 4 mg in saline) and observed for delivery times. Other rats were treated as above or by intrauterine infusion of P4 (soluble P4, Sigma-Aldrich Co., various doses) or vehicle through a cannula inserted into the uterine lumen next to electrodes attached to one horn of the uterus (ca. 3 fetuses anterior to the cervix) after ketamine + xylazine anesthetic on day 22 and EMG was measured for 4 to 8 hrs. Cervical collagen was estimated by light-induced fluorescence (LIF in photon counts). Statistical differences were assessed by one-way ANOVA and the Student's t-test. **Results:** P4 (1 and 2 days, s.c.) prevents delivery (P4-treated rats sacrificed on day 25) and does not effect ($P>0.05$) the already low cervical LIF (ca. 500 photons for CTL and P4-treated rats). During recording complete delivery of the fetuses occurs in some CTL rats. P4 (s.c.) significantly reduces the EMG burst frequency (bursts/30 min ± SD: 1 day =11.1 ± 1.7 vs. CTL 23.2 ± 3.5, $P<0.01$; 2 day =10.5 ± 1.3 vs. CTL 25.4 ± 4.4, $P<0.01$). The EMG burst amplitudes are also significantly lower in the P4-treated animals (μV ± SD: 1 day = 74 ± 8 vs. CTL 280 ± 58, $P<0.008$; 2 day = 127 ± 31 vs. CTL 230 ± 14, $P<0.02$). Thus the mean burst integrals (V^2) are suppressed at 1 ($P<0.001$) and 2 ($P<0.002$) days after P4 treatment vs. CTL, but not the burst duration ($P>0.05$, ca. 30 seconds). P4 infusion, even doses equal to 2 mg, fails to inhibit EMG activity ($P>0.5$). However, nifedipine (10 μg) and $MgSO_4$ (1.8 mg) infusion, but not indomethacin (up to 1000 μg), immediately ($P<0.001$) decrease EMG. **Conclusions:** 1) P4 inhibits delivery by suppression of myometrial EMG and contractility when the cervix is already ripened. 2) P4 effects require longer time (ca. 24 hr) perhaps due to genomic actions. 3) P4 has little direct nongenomic action on EMG. 4) Nifedipine and MgSO4, but not indomethacin, rapidly suppress EMG.

T-223

Suppression of the SUMO Pathway in Human Myometrium Modulates PR and NF-kB Signalling. Draga Tchipeva,[1] Anysia Semertzidou,[1] Jordan Read, Junichi Takahashi,[1] Sung H Kim,[1] Mark Johnson,[1,2] Phillip R Bennett,[1] Vasso Terzidou.[1,2] *[1]Parturition Research Group, IRDB, Imperial College, London, United Kingdom; [2]Department of Obstetrics & Gynaecology, Chelsea & Westminster Hospital, London, United Kingdom.*

BACKGROUND: Sumoylation is a post-translational modification that modulates protein function by altering protein structure, stability, protein-protein interactions and subsequently cellular localization. It is thought that NF-κB signalling and the progesterone receptor (PR) are central factors in human labour and are highly regulated by covalent binding to SUMO (small ubiquitin related modifier). NF-κB is a family of transcription factors, which regulate many pro-labour genes, release of chemokines, inflammation in the myometrium and cervix, and prostaglandin synthesis. PR promotes myometrial quiescence and inhibits contractility.

AIM: To examine whether sumoylation can be suppressed in human myocytes and investigate its role in PR and NF-κB pathways.

METHODS & RESULTS: Western blot analysis revealed that IL-1β increases global sumoylation in myometrial cells. In primary human myocytes Gingkolic acid (GA) significantly suppressed sumoylation both basally and after IL-1β treatment. Although GA does not inhibit Iκ-Bα degradation in IL-1β induced cells, it suppresses expression of PR in basal and IL-1β induced myometrial cells. In human pregnant myocytes PR-A is subject to sumoylation by SUMO-1 and PR-A SUMO-1 modification is augmented in response to MPA and further enhanced if combined with IL-1β treatment. We have shown that GA significantly suppresses the basal mRNA expression of a number of genes regulated by PR such as MMP1, MMP3 and MMP10 and also significantly decreases the IL1-b induced expression of NF-kB regulated genes such as IL-8 and COX-2.

CONCLUSIONS: Our results suggest that SUMO modification is intricately associated with the process of human parturition and suppression of sumoylation in human myocytes modulates PR and NF-kB signalling resulting in changes in expression of PR and NF-kB regulated genes.

T-224

Regulation of Sumo-Pathway Enzymes upon Inflammatory Stress and Labour. Junichi Takahashi,[1] Sung H Kim,[1] Anysia Semertzidou,[1] Jordan Read,[1] Draga Tchipeva,[1] David A MacIntyre,[1] Jan Brosens,[2] Phillip Bennett,[1] Vasso Terzidou.[1,3] *[1]Parturition Research Group, IRDB, Imperial College, London, United Kingdom; [2]Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom; [3]Obstetrics and Gynaecology, Chelsea & Westminster Hospital, London, United Kingdom.*

Background: Sumoylation functionally modifies proteins by altering stability, protein-protein interactions, and cellular localization. Two key regulators of the onset of parturition, NF-κB and PR, are both regulated by covalent binding of SUMO. PIAS proteins are E3 SUMO ligases and act as transcriptional co-regulators. We have previously reported dynamic changes in myometrial hypersumoylation with labour and in response to inflammatory stress.

Aim: To elucidate the regulation of the SUMO-pathway enzymes upon inflammatory stress and labour.

Materials and Methods: Human myometrial biopsies were obtained at caesarean sections before (L-) or after (L+) labour onset (n=6, each group). The difference in expression of each sumoylation cycle enzyme between L+ and L- was analyzed by Q-RT PCR and Western blotting. To assess the effect of inflammatory stress on each enzyme primary myocyte cultures were established and time course experiments were performed with IL-1β and mRNA and protein expression was determined. Primary myocytes were transiently transfected with an NFBG reporter vector and PIAS1 or PIAS3 expression vectors to evaluate their effect on NF-kB activity.

Results: We have found a significant increase in RanBP2, SENP2, PIAS3, SAE1 and SAE2 mRNA in human myometrium after the onset of labour whereas PIAS1 and Ubc9 expression remained unchanged (n=6 each group; $p<0.05$). Consistent with changes of expression with labour, IL-1β increases SUMO-1 conjugates in primary myocytes and significantly increases expression of RanBP2, SENP2, PIAS3, SAE1 and SAE2, whereas PIAS1 and Ubc9 expression remains unchanged. Using transient transfections we have shown a regulatory link between PIAS3 (but not PIAS1) overexpression and NF-κB reporter activity resulting in up-regulation of the basal NF-kB activity.

Discussion: The increase of SUMO-1 modification with labour is associated with an upregulation of key SUMO-enzymes with labour and inflammation. Establishing the exact mechanisms by which sumoylation modulates NF-κB signalling and PR, may provide a platform for the use sumoylation inhibitors against pre-term labour.

T-225

Differential Expression of Toll-Like Receptor 10 and Interleukin-13 in Term Pregnant Myometrium and Their Regulation by Vitamin D in Human Uterine Smooth Muscle Cells. Chandrasekhar Thota,[1] Waseem Khoder,[1] Robert E Garfield,[2] Ayman Al-Hendy.[1] *[1]Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Obstetrics and Gynecology, St.Joseph Hospital and Medical Center, Phoenix, AZ, USA.*

BACKGROUND: Toll-like receptors (TLRs) activate immune response during infection**.** African Americans have been reported to have higher basal inflammation, lower levels of serum vitamin D, a ligand with anti inflammatory properties, and higher incidence of preterm birth. Literature suggests that single nucleotide polymorphisms in TLR10 and IL-13, an immunoregulatory cytokine, are associated with preterm birth. However, literature on the expression of TLR10 and IL-13 in term myometrium and the role of vitamin

D in the regulation of TLR10 and IL-13 expression in uterine smooth muscle (UtSM) cells, in an inflammatory environment caused by infection, are lacking.
OBJECTIVE: To assess the expression of TLR10 and IL-13 in myometrium obtained from term pregnant women not-in-labor (TNL) and women who had a trial of labor, and to assess vitamin D regulation of IL-13 and TLR10 expression, *in vitro*, in UtSM cells cocultured with monocytes to simulate inflammatory conditions.
METHOD: Myometrium tissues (0.5cm) were collected from TNL subjects (n=6) and women who had a trial of labor (n=6) to assess the mRNA expression of TLR10 and IL-13. To assess if vitamin D regulates the expression of TLR10 and IL-13 in an inflammatory environment, UtSM cells were cocultured with monocyte lineage (THP1 cells; 200k) cells and treated in triplicates with vitamin D (5, 50 and 150nM) for 24h. Expressions of TLR10 and IL-13 were measured by real time PCR.
RESULTS: Expression levels of TLR10 and IL-13 mRNA were higher in myometrium tissues obtained from TNL subjects compared to the levels in tissues obtained from subjects in labor. Vitamin D treatment caused a significant decrease ($p<0.05$) in THP1 induced TLR10 and increase ($p<0.01$) in IL-13 in UtSM cells.
CONCLUSIONS: High expression of TLR10 in term pregnant women not-in-labor subjects indicates a role for TLR10 in the maintenance of pregnancy. As the process of contractions is not initiated in term pregnant women not-in-labor subjects, IL-13 levels remain significantly higher compared to laboring tissues indicating a role for IL-13 during labor. Results from *in vitro* co-culture studies suggest that vitamin D plays a role in the regulation of TLR10 and IL-13 in uterine smooth muscle cells.

T-226

Maternal Race and Optimal Gestational Age for Elective Repeat Cesarean Delivery. Gustavo A Vilchez, Robyn Roberts, Anushka Chelliah, Zareen Amin, Jennifer Bruni, Michael Kruger, Ray O Bahado-Singh. *Department of Obstetrics & Gynecology, Wayne State University, Detroit, MI, USA.*
Background: Hospital-based studies report an increased risk of newborn prematurity-related complications (PRC) with elective repeat cesarean delivery (ERCD) between 37 to <39 weeks gestation. There is absence of epidemiologic level data that would reflect the risks in diverse clinical settings throughout the US. Further, the impact of maternal race on neonatal morbidities due to borderline prematurity is unclear.
Objective: To perform a population-based study to determine risks of PRC due to ERCD between 36-39 weeks and the effect of maternal race on these risks.
Study Design: The National Center for Health Statistics-CDC Natality Database for U.S. from 2004-2008 was reviewed. Only states reporting data on ERCD were included in the analysis. Exclusion criteria used were: multiple pregnancy, attempted trial of labor, fetal anomalies, and diabetes and/or hypertensive disorders including eclampsia. The odds ratio (OR) for PRC defined as low Apgar scores, assisted ventilation, admission to intensive care unit, surfactant use, antibiotic use and neonatal seizures, was determined. Forward-stepwise regression analysis standardized for confounders was performed to calculate the adjusted OR (95% CI) of PRC based on gestational age (GA). Cases delivered from 36-39 weeks were studied with 39 weeks serving as the reference group.
Results: A total of 930,421 ERCD were performed in the reporting states in the U.S. between 2004-2008. After exclusions, 699,051 had ERCD between 36-39 weeks. For the overall population, the rates of major PRC such as NICU admit, prolonged ventilation and need for surfactant were significantly higher in newborns ≤38 weeks compared to 39 weeks GA ($P<0.001$). Similar findings were found in sub-analysis of whites. For African Americans (AA) the rate of PRC was not significantly different at 38 compared to 39 weeks.
Conclusion: We report increased rates of PRC in ERCD before 39 weeks for the U.S. population, similar to that found in smaller hospital-based studies. However, for AA newborns there was no increase in major PRC at or after 38 weeks suggesting earlier maturation of these fetuses.

T-227

Epigenetic Regulation of Matrix Metalloproteinases and Their Inhibitors in Parturition. Zoe L Vincent,[1,2] Murray D Mitchell,[1,2,3] Anna P Ponnampalam.[1,2] *[1]The Liggins Institute, The University of Auckland, Auckland, New Zealand; [2]National Research Centre for Growth and Development, The University of Auckland, New Zealand; [3]Centre for Clinical Research, University of Queensland, Brisbane, Australia.*
Introduction: Matrix metalloproteinases (MMPs) modulate the tensile strength of the fetal membranes and play key roles in parturition; particularly the rupture of membranes and placental detachment from the uterus. Abnormal activation of MMPs (such as during infection) and subsequent degradation of the membranes are thought to contribute to preterm birth. Despite the mounting evidence of the epigenetic control of MMPs and membrane-bound MMPs (MT-MMPs) in other tissues, little is known about their epigenetic regulation in gestational tissues (GT) under normal and pathological settings.
Objectives: To characterise MT-MMP expression in term GTs and to investigate the regulation of expression and activity of MMPs and MT-MMPs by DNA methylation and infection in human GT explants.
Methods: MT-MMP gene expression in pre-labour term GT was quantified using real-time PCR. MMP activity was measured by gelatin zymography. GT explants isolated from pre-labour term placentae were used to determine the effect of methylation inhibition by 5-aza-2'-deoxycytidine (AZA) and/or infection by LPS treatments.
Results: Our results show for the first time that there is a tissue specific labour effect on the expression of MT1-MMP and MT5-MMP in gestational tissues. MT1-MMP expression was seven times higher post-labour compared to pre-labour villous tissues ($P\leq0.05$), while MT5-MMP expression was over seven times higher prior to labour in amnion and choriodecidua. Consistent with the characterisation data, combined AZA and LPS treatment caused a 1.9 fold increase of MT1-MMP expression in villous tissue ($P=0.06$) and over three fold increase in amnion ($P\leq0.05$). In contrast, MT5-MMP expression showed a two fold decrease in amnion ($P=0.06$) and a five fold decrease in choriodecidua ($P\leq0.05$) following the treatment. Furthermore, MMP-2 activity was increased over three fold in villous tissue ($P\leq0.05$) following AZA treatment.
Conclusion: Our data suggest that MMPs and MT-MMPs could be regulated by DNA methylation in human placenta, thus contributing to the timing of labour. The results also imply that there are tissue specific differences in the regulation of MMPs and also that there could be many feedback regulatory mechanisms in response to these treatments on individual MMP transcription.

T-228

Expression of CRH/Urocortin Gene Family in Mouse Gestational Tissues during Late Pregnancy. Sharon Battersby,[1] Chiara Voltolini,[2] Felice Petraglia,[2] Jane E Norman.[1] *[1]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy.*
Background & Aims: There is increasing evidence for a role for the CRH/urocortin family of genes in human pregnancy. CRH has been implicated in the initiation of human parturition and there is evidence that CRH and the urocortins are involved in spontaneous preterm labour. However, little information is available on the presence or function of the CRH/urocortin genes in pregnancy in the mouse. The aim of the present study was to investigate the expression of CRH, the urocortins and their receptors in mouse utero-placental tissues during late pregnancy.
Methods: All animal care and experimental protocols were approved by the appropriate animal ethics authorities. Placental, uterine and fetal membrane tissues were separated and collected from timed-pregnant mice on days 16-19 of pregnancy (N=10 for each group; parturition occurred on D20). RNA was extracted from the tissues and Taqman real-time PCR was performed using standard techniques.
Results: CRH and urocortin1 were expressed at low levels in the murine placenta and did not vary significantly during late pregnancy. Urocortin2 was the most abundant urocortin in mouse placenta and levels increased significantly with advancing pregnancy from D16 to D19 ($p<0.001$). Urocortin2 levels were lower in fetal membranes, but also increased significantly on D19 of pregnancy ($p<0.05$ D19 vs D16, D17, D18). In contrast, urocortin3 concentrations were higher in fetal membranes than in placenta and decreased significantly on D17-19 compared with D16 ($p<0.05$). CRH-R1 was predominantly expressed in placenta and concentrations did not vary significantly. However, CRH-R2 was more widely expressed and the level in the fetal membranes increased significantly on D18 compared with D16 ($p<0.05$).
Comments: These data indicate that urocortin2 is the major urocortin in mouse gestational tissues, with the up-regulation in placenta and fetal membranes in late pregnancy suggesting a role for the gene in parturition. Moreover, the co-incident decrease in urocortin3 in fetal membranes suggests that the balance between the two urocortins, which both act via CRH-R2, may be important in their role in mouse pregnancy and parturition.

T-229

Serum Progesterone Concentrations in Women with a Previous Preterm Birth Treated with Vaginal Progesterone Supplementation. Manju Chandiramani,[1,2] Paul T Seed,[1] Phillip R Bennett,[2] Andrew H Shennan,[1] Rachel M Tribe.[1] *[1]Division of Women's Health & Women's Health Academic Centre KHP, King's College London; [2]Institute of Reproductive & Developmental Biology, Imperial College London.*

Background: Progesterone is used for the prevention of spontaneous preterm birth (SPTB). It is proposed to promote maintenance of pregnancy via its anti-inflammatory properties and actions on prostaglandin synthesis and uterine smooth muscle.

Objective: We determined if women at high risk of cervical shortening and SPTB have lower serum concentrations of progesterone, and whether vaginal progesterone supplementation influences circulating concentrations as pregnancy progresses.

Methods: Serum progesterone was measured using ELISA immunoassay in longitudinal samples (n=226) obtained from 64 women (14-28 weeks) with a history of at least one previous preterm delivery. Women were recruited as part of a prospective study which assessed transvaginal cervical length every two weeks. If the cervix shortened (≤25 mm), women were randomly assigned to cerclage or progesterone. Concentrations of progesterone were measured in longitudinal serum samples. Data were log-transformed, analysed using STATA, and results expressed as geometric means and ratios (95% confidence intervals; CI).

Results: Thirty-six percent (23/64 women) delivered preterm (<37 weeks; mean of 3.7 samples/woman) compared with 41 women who delivered at term (mean of 3.4 samples/woman). Baseline serum progesterone concentrations (14-18 weeks) in both groups were similar (36.1 versus 39.7ng/ml in preterm compared to term; ratio 0.90,95%CI 0.75-1.08;p=0.27). Concentrations in women destined to develop a short cervix were similar to those who did not exhibit cervical shortening (ratio 0.98,95%CI 0.84-1.15;p=0.83). Randomisation to vaginal progesterone supplementation had little effect on concentrations (15% increase, 95% CI 0.98-1.35;p=0.09). There was no significant difference in concentrations between women who delivered before 34 weeks (effect 0.89,95%CI 0.74-1.06;p=0.19) and 37 weeks (effect 0.92, 95%CI 0.79-1.09;p=0.34) to those who delivered at term.

Conclusions: Serum progesterone concentrations were similar in women regardless of cervical shortening, gestation at delivery or treatment. Any beneficial effect of vaginal progesterone supplementation, therefore, is more likely to be mediated via local effects on the cervix.

Funding: Action Medical Research (Grant SP4113) and Tommy's the Baby Charity.

T-230

Morphological and Molecular Basics of Uterine Scar Dehiscence in Women with Undifferentiated Connective Tissue Dysplasia. Tatiana A Demura, Evgeniya A Kogan, Marina I Kesova, Nataliya E Kan, Andrey E Donnikov, Gennadiy T Sukhikh. *Anatomic Pathology, Research Center of Obstetrics Gynecology and Perinatology.*

Undifferentiated connective tissue dysplasia (uCTD) is characterized by connective tissue hyperextensibility, resulting in its relative weakness and development of several pathological conditions, like mitral valve prolapse, varicouse vein disease, myopia, skeletal pathology and etc.. Our previous studies showed higher frequency of complications during pregnancy and labour in women with uCTD, especially with uterine scars that frequently develop dehiscence. We suspect uCTD is a genetically based disorder involving extracellular matrix disorganization. The objective of the study was to investigate morphological, immunohistochemical and molecular basics of uterine scar dehiscence in women with uCTD. Materials and methods: 90 pregnant women with uterine scar were recruited in the study (age from 18 to 40 yy). They were divided into 2 groups: with uCTD and without (45 contra 45). The biopsy samples of uterus with scar and adjacent tissue were taken during caesarian section. IHC reactions were performed on paraffin sections with antibodies to ER α and β, collagen -III, -IV, MMP9 and VEGF (all Lab Vision) and antibody of Mg2+ channels TRPM7 (Abcam). Molecular-genetic analysis was made using fluorescent hybridization probes and melting curve analysis to detect ER alpha (ESR1) and ER beta (ESR2) polymorphisms, and MMP9 and VEGF gene polymorphisms. Reaction results were evaluated with the help of quantitative and semiquantitative methods and statistic analysis. Results: the majority of women with uCTD developed uterine scar dehiscence that is based on connective tissue disorganization: mucoid and fibrinoid changes. uCTD is characterized by disturbed accumulation of laminin and collagen III and IV and relative decrease in VEGF which may result in microcirculation disturbancies. As ER alpha it was very low in both groups, whereas ER beta, MMP9 were rather high in the group with uCTD. Interestingly, TRPM7 was rather low in women with uCTD. Molecular analysis showed gene polymorphism of ERs, VEGF, MMP9 in patients with uCTD. Conclusion: Obtained results show that uCTD is characterized by connective tissue disorganization that might be genetically determined and is based on extracellular matrix and microcirculation disbalance and on impaired regeneration. uCTD may be diagnosed and thus uterine scar dehiscence in women may be predicted.

T-231

Characterization of Gestationally Regulated Estrogen Receptor α (ESR1) Isoforms in the Pregnant Myometrium. Lee R Sang, Robert C Moore, Christina Kachulis, Arvind Suresh, Evan Easton, Steven N Caritis, Pancharatnam Jeyasuria. *Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA.*

Estrogen (E2) signaling through its receptor (ESR1, ERα) is thought to regulate the timing of parturition by increasing the expression of contractile associated proteins (CAPs) and inhibiting PR transactivation. We hypothesize that alternate ERα transcripts in the pregnant uterus play a critical role in governing both ERα66 and PR responsiveness.

Previous studies have identified both ERα46 and ERΔ7 as potential modifiers of estrogen and ERα66 action. The initial ERα66 knockout mice showed partial estrogenic responsiveness due to ERα46 persistence. Utilizing human myometrial tissues isolated from term and pre-term non-laboring patients, the hTERTHM cell line and a gestational series of pregnant mouse uteri, we identified the differential gestational expression of two splice variants (ERα46 and ERΔ7) and the full-length ERα66.

In both human and mouse uteri, ERα46 is the dominant nuclear isoform but as term approaches is replaced by ERα66. Diminished nuclear levels of the ERΔ7 also occur as term approaches in human myometrium. These parallels in the mouse and human suggest that ER isoforms mediate differential actions in the pregnant uterus and play temporal as well as spatial roles in pregnancy. We have also determined that the estrogen responsiveness of ERα46 in primary human myometrial explant cultures differ significantly between myometrium isolated from non-laboring preterm and term patients. Increased levels of nuclear ERα46 result upon exposure to E2 in term patients whereas in preterm myometrium the 46 kDa isoform level remains unresponsive to E2.

Utilizing siRNA knockdown, RTPCR and RPA we have confirmed the presence of these ERα isoform transcripts in the human and mouse pregnant myometrium. Utilizing siRNAs we demonstrate independent down regulation of both the ERα66 isoform and the ERα46 isoform in the hTERTHM cell line. siRNA knockdown also reveals that ERα46 can mediate E2 activated Connexin 43. RPA analysis confirms decreased ERΔ7 mRNA levels at term, which correlates to protein levels. We postulate that ERα46 has a transcriptional role albeit different from that of ERα66 and that both ERα46 and ERΔ7 block ERα66 nuclear receptor function. ERα66 becomes the dominant isoform at term, probably through PR de-repression, up regulating CAP expression, resulting in the onset of labor.

T-232

Mechanical Stretch Induces Pro-Inflammatory Cytokine Expression and Leukocyte Transendothelial Migration into the Myometrium. Yu-Hui Lee,[1,2] Oksana Shynlova,[2] Stephen Lye.[1,2,3] *[1]Physiology, Univ. Toronto; [2]Ob/Gyn, Univ. Toronto; [3]Samuel Lunenfeld Res. Inst, Mount Sinai Hospital, Toronto, Canada.*

Spontaneous labour at term is associated with increased cytokine production, adhesion molecule expression and leukocyte invasion into the myometrium. We previously demonstrated an association between uterine occupancy, increased myometrial production of MCP-1, concurrent macrophage influx into the rat myometrium and the initiation of labour in vivo. We hypothesize that mechanical stretch induces pro-inflammatory cytokine secretion by human myometrial smooth muscle cells (SMC) which facilitates macrophage/neutrophil transendothelial migration (TEM) into the myometrium via upregulation of adhesion molecules and/or enhancement of their migratory characteristics. Methods. 1) To test this hypothesis we cultured human myometrial SMC line (hTERT-HM) on flexible-bottomed collagen I-coated culture plates and applied static mechanical stretch using the Flexcell-5 strain unit for 24 hours (h) with or without specific intracellular signaling inhibitors. Stretch-conditioned media (SCM) and total RNA were collected and analyzed with multiplex human cytokine assays (Bio-Rad) and Real-Time PCR respectively. 2) Next we stimulated human uterine myometrial microvascular endothelial cells (UtMVEC-Myo) with SCM for various time intervals (up to 12h) to investigate mRNA expression of adhesion molecules (ICAM-1,

VCAM-1 and PECAM-1). 3) Adhesion assay and TEM assay were performed by seeding UtMVEC-Myo cells onto gelatin-coated 96-well plate or 3-8-μm transwell inserts to examine whether stretch-induced cytokines promote the adhesion and TEM of primary human neutrophils and cultured macrophages. Results. Bio-Plex screen revealed cytokines (IL-6, IL-8, VEGF, GRO-α) whose levels were significantly elevated by stretch. Results indicated that: 1) PKC and p38 MAPK mediate stretch-regulated cytokine production by hTERT-HM; 2) VEGF acts as an endothelium activator, whereas 3) IL-8 and GRO-α operate as leukocyte recruiters. 4) ICAM-1 and VCAM-1 mRNA expression increases significantly after 2-4h stimulation with SCM or VEGF. 5) TEM assay showed significant increase in neutrophil migration toward IL-8 and GRO-α stimuli. Overall these results support our hypothesis that mechanical stretch can induce cytokine secretion capable of promoting peripheral leukocyte entry into the myometrium which, in turn, promotes a physiologic inflammation and the onset of labour.
Funding: CIHR (MOP-37775)

T-233

Questioning the Role of Oxytocin in Parturition: Onset of Spontaneous Labor in 3 Women with Panhypopituitarism. Sharon Maslovitz, Guy Bibi, Avital Skornick-Rapaport, David Pauzner, Michael Shenhav, Ariel Many, Joseph B Lessing. *Obstetrics and Gynecology, Lis Maternity Hospital, Sourasky Medical Center, Tel Aviv, Israel.*
Objective: Oxytocin is a nanopeptide hormone secreted by the posterior pituitary gland with a well established uterotonic activity and a short half life. Its role in initiating the vigorous and regular contractions typical of the first stage of spontaneous labor is still controversial. We report three cases of pregnant women with panhypopituitarism who had spontaneous onset of labor, undermining the role of oxytocin (lacking in panhypopituitarism) in the first phase of labor.
Methods: Three women with no residual pituitary function conceived through ovulation induction and were treated with thyroid replacement therapy, desmopressin and steroids throughout gestation. Case 1 had childhood hypophysectomy due to a large tumor. Case 2 suffered from destructive hypophysitis following previous delivery and case 3 had no pituitary function due to previous radiation therapy to the region. In all three cases no detectable pituitary hormones were found in repeated blood tests. We report the course of pregnancy and delivery in the lack of natural or added oxytocin.
Results: All three pregnancies were uncomplicated and progressed to full term with hormonal replacement as stated above. Case 1 had an uncomplicated spontaneous delivery at 39 weeks without any substitution of oxytocin before, during or after delivery. The onset of delivery was spontaneous for case 2 as well but she had a cesarean section due to non-reassuring fetal heart rate tracing while she was fully dilated and the vertex at the spines. Case 3 had spontaneous labor and delivery without any pharmacological intervention. Lactation did not ensue in all three cases. Post-partum uterine atony mandated the administration of 600mcg misoprostol for case 3.
Conclusions: Maternal pituitary oxytocin is not obligatory for uterine activation in the first phase of parturition.

T-234

Guinea Pig Parturition Occurs in the Absence of Serum Progesterone (P4) Withdrawal yet Anti-Progestin Treatment Induces Preterm Labor: A Model for Human Parturition. WC Tong,[1] X Fang,[2] GN Europe-Finner,[1] MJ Taggart,[1] BF Mitchell.[2] *[1]Institute of Cellular Medicine, Newcastle University, United Kingdom; [2]Obnstetrics and Gynecology, University of Alberta, Canada.*
Objectives: Maternal serum progesterone (P4) withdrawal is a critical step in the initiation of parturition in mice and rats but no such fall in P4 prior to labor has been reproducibly reported for humans. Although now less commonly used than mice and rats as an experimental model of parturition, recent interest has been sparked in re-investigating the applicability of the guinea pig as a model of human parturition. Our aims in this work with time-mated pregnant guinea pigs were two-fold. First to monitor maternal serum progesterone levels in advance of term parturition. Second, to explore the effects of the P4 antagonist RU486 in the instigation of preterm labor.
Methods: Serial maternal serum samples were obtained using indwelling jugular catheters or venipuncture beginning at d30. Preterm labour was induced using RU486 (RU, 3 mg/kg body weight) on gestation d 55 and 56 (~0.8 of term, analogous to ~32 weeks human gestation. If parturition wasn't imminent by 48h, a single dose of oxytocin (OT, 2 U/kg) was given. Control groups received vehicles only. Sixty time-mated guinea pigs were used in these studies.
Results: Spontaneous delivery occurred at d 68.2±0.3 d (mean ± SEM; n=12). Median litter size was 4. Of 44 pups, 42 appeared healthy and 2 were either stillborn or died shortly after birth. Serial serum samples were obtained from 39 animals in total. Peak P4 levels occurred between 30-35d (522±80 ng/ml) then declined to d55-56 (167±40 ng/ml). Thereafter, there was a slight increase. P4 levels within 24h of parturition were 205 ± 35 ng/ml. In 6/11 RU-treated animals, delivery occurred within 48h after the first injection. In 4 animals, OT was administered after 48h and delivery occurred within 12h. 38 of 39 preterm pups did not survive. Only 1 animal receiving RU did not deliver preterm. Total success rate for this model of preterm birth was 91%. None of the 5 controls developed preterm birth.
Conclusions: Birth in guinea pigs is not preceded by a precipitous decline in maternal serum P4. However, preterm birth can be induced consistently with an anti-progestin. This suggests that there may be a 'functional withdrawal' of P4 in this species, in keeping with the suspected mechanism evoking human parturition.
Supported by MRC (UK, G0900525 and G0902091) & AIHS & WCHRI (Canada).

T-235

Proteomics Analysis of Estrogen Responsive Mitochondrial Protein *S*-Nitrosylation in Human Umbilical Cord Vein Endothelial Cells. Seiro Satohisa,[1] Hong-hai Zhang,[1] Ying-ying Yang,[1] Lan Huang,[2] Dong-bao Chen.[1] *[1]Dept of Ob/Gyn, Univ of CA, Irvine, CA, USA; [2]Physiol & Biophy, Univ of CA, Irvine, CA, USA.*
Introduction: S-nitrosylation (SNO) is an emerging pathway crucial for nitric oxide (NO) to exert its cellular function. We have recently shown that estrogens stimulate dynamic protein SNO in endothelial cells. Mitochondria are known to be the primary subcellular organelle that NO targets in many cells including endothelial cells. However, the effects of estrogens on global mitochondrial protein SNO are unknown. **Objectives**: to determine if estrogen stimulates mitochondrial protein SNO and if yes, to identify the estrogen responsive mitochondrial *nitroso*-proteins. **Methods**: Human umbilical cord vein endothelial cells (HUVEC) were treated with estradiol-17β (E2, 10 nM) or nitrosoglutathione (GSNO, 1mM) for 20 min. The cells were fixed and SNO-proteins were labeled by biotin switch (BST) and visualized with fluorescently labeled subcellular organelle (Golgi, Endoplasmic reticulum, mitochondria and nucleus) trackers by immunofluorescence microscopy. Mitochondria were purified using the magnetic microbeads coated with anti- TOM22 antibody and verified by immunoblotting of marker proteins. Mitochondrial proteins were subjected to BST for labeling SNO-proteins. Levels of SNO proteins were determined by immunoblotting with anti-biotin antibody. The biotin-labeled mitochondrial SNO proteins were tryptically digested for capturing the biotin-labeled SNO-peptides using avidin-coated beads and identified by Mass Spectrometry. **Results**: Treatment with E2 and GSNO significantly increased the levels of mitochondrial SNO-proteins. Mitochondria were labeled *in situ* with the greatest protein SNO response to estrogen and GSNO, whereas the other organelles were with weaker SNO signals. Proteomics analysis of the SNO-peptides identified 13, 46 and 76 SNO-proteins in the mitochondrial proteomes in control, E2- and GSNO- treated HUVEC, respectively. Function analysis suggested that SNO-proteins are associated with various mitochondria functions, including apoptosis, energy, translational machinery and protein import, redox and calcium as well as iron homeostasis, *etc*. **Conclusion**: Estrogen rapidly stimulates protein SNO in endothelial mitochondria, implicating mitochondrial protein SNO to be crucial for mediating the vasoprotective effects of estrogens (Supported by HL70562 & HL98746).

T-236

Inflammatory Cytokines and Cervical Length: Relationship to Incidence of Preterm Birth. Monica Smith,[1] Dara Seybold,[2] Kenneth Aladefa,[2] Jamie L Miller,[3] Byron C Calhoun.[1] *[1]Department of Obstetrics and Gynecology, West Virginia University Physicians of Charleston, Charleston, WV, USA; [2]Center for Health Services & Outcomes Research, Charleston Area Medical Center Health Education and Research Institute, Charleston, WV, USA; [3]Director, Life Science Division, Mid-Atlantic Technology, Research & Innovation Center, Morgantown, WV, USA.*
Objective: Levels of interleukin (IL) 1 alpha, 1 beta, IL-4, IL-6, IL-10, and IL-13 in low risk population coupled with transvaginal cervical length help predict spontaneous pre-term birth.
Design: Prospective pilot study of 39 patients between 12 0/7 and 31 6/7 weeks gestation presenting for initial prenatal care. Vaginal swabs from each patient

evaluated for IL-1 alpha, IL-1 beta, IL-6, IL-8, IL-10 and IL-13. Concentrations based upon gene and receptor status. Assays performed in a laboratory by single operator blinded to clinical status. Transvaginal ultrasounds performed with cervical length.

Materials and Methods: Patients followed prospectively for spontaneous preterm delivery (<37 weeks gestation). Mean cytokine levels compared between preterm/term birth groups and infection/no infection groups with Student t-test. Cytokine levels tested to evaluate correlation with shorter cervical lengths using Pearson's correlation. P values <0.05 considered significant.

Results: Of the 39 patients enrolled, 8 (20.5%) delivered pre-term. Infection was associated with spontaneous pre-term birth, infection rate at 75% versus 33% for term birth (p=0.045). No patients had shortened cervixes ≤30 mm, while 9.68% had measurements ≤ 35mm. Mean IL-1alpha, IL-4, IL-10 and Il-13 levels were not statistically different between pre-term and term births. However, IL-1beta and IL-6 levels were significantly lower in preterm birth group (6.28 pg/ml ±12.7and 3.96 pg/ml±3.8) compared to term births (25.41 pg/ml ±41.6; p=0.035and 35.7 pg/ml ±55.3; p=0.003). There was moderate correlation between IL-10 and cervical length (r=0.54; p=0.002). IL-1alpha, IL-1beta, IL-6, IL-10 and IL-13 had no correlation with cervical length. Cytokine concentrations were not associated with infection.

Conclusions: Unlike previous reports, IL-1beta and IL-6 levels were lower in our preterm birth patients. IL-10 may provide a moderating effect on both inflammatory cytokines and affect cervical length by its anti-inflammatory action. Further study with larger numbers of patients is warranted.

T-237

The Effect of Pregnancy on Cardiac Chemokine Gene Expression in Mice.

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Introduction: Increased cardiac output is a well known phenomenon that occurs during pregnancy and the heart normally compensates by developing reversible physiological hypertrophic growth. A pathological model of hypertrophy was developed in mice to act as a positive control in order to study cardiac functions in pregnancy. Chemokines are potent chemotaxins involved in neutrophil activation and have been identified as critical factors during different stages of pregnancy. We aimed to study chemokine gene expression in the hearts of non-pregnant and pregnant mice.

Methods: 3 groups of mice were used: non-pregnant (n=8), late pregnant (n=10) and mice subjected to transverse aortic constriction to act as a pathological model of hypertrophy (n=12). Left and right ventricles were excised, placed in liquid nitrogen and stored at -80^{0}C. Total RNA was extracted from these tissues using the miRNeasy kit from Qiagen and the mRNA converted to cDNA. Copy numbers of atrial nutriuretic peptide (ANP), brain nutriuretic factor (BNF), beta-myosin heavy chain (β-MHC), CCL2, CCL5, CCL20, CXCL1, CXCL5 and GAPDH were measured by qPCR using a Rotor-Gene™(Corbett Research, Australia).

Results: Aortic constriction increased ANP, BNF and β-MHC by 493.4, 641.2 and 982.4% in the right ventricle and by 2591.2, 437.1 and 943.2% in the left ventricle respectively. Pregnancy did not change the levels of these genes in the right and the left ventricle. The chemokines measured were not affected by aortic constriction in either the two chambers of the heart. No changes in CCL2, CXCL1 and CXCL5 were observed in pregnancy. However, for both CCL5 and CCL20 decreases in gene expression were seen in the right ventricle (by 53.0% and 97.4% respectively) in the left ventricle (by 54.3% and 95.7% respectively). All significant changes reported are p<0.05.

Conclusions: These data show all the chemokine genes studied are expressed in the ventricular compartments of the mouse heart. The traditional markers associated with cardiac hypertrophy are not upregulated by the physiological hypertrophic growth that occurs during pregnancy. CCL5 and CCL20 are decreased during pregnancy and the roles of these chemokines in the heart require further elucidation.

T-238

CRTH2 Expression in Peripheral Blood Mononuclear Cells Increases in Pregnancy but Does Not Result in an Increase in the Production of the T Helper 2 Cytokine IL-4.

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Intrauterine inflammation and/or infection is strongly associated with preterm labour. Inflammation and infection are associated with T helper 1 pro-inflammatory cytokines, which can alter the bias from the pro-pregnancy T helper 2 cytokine profile. We examined the changes in T helper 2 lymphocytes throughout pregnancy in maternal peripheral blood using Chemoattractant Receptor Homologous to T helper 2 cell (CRTH2) expression a marker of T helper 2 lymphocytes. The functional profile of CRTH2 positive lymphocytes was determined by their production of IL-4, an interleukin that regulates the Th1:Th2 balance. The effect of CRTH2 agonists 15dPGJ2 and Pyl A on IL-4 production was examined.

Blood was collected at different stages of pregnancy, with non pregnant women serving as a control. For interleukin studies cells were cultured overnight, stimulated with PMA/Ionomycin with or without pre incubation with CRTH2 agonists. Flow cytometry was used to determine CRTH2, CD4, and intracellular IL-4. SPSS v18 was used for statistical analysis.

CRTH2 expression increases in pregnancy from 1.79% in non pregnant women (n=10) to 1.88% at 28 weeks (n=10), and 2.15% at term (n=9). CRTH2 expression was reduced from 1.81% in term labouring women (n=10) to 1.14% in preterm labouring women (n=3) p=0.04. PMA/Ionomycin stimulated IL-4 production in CRTH2 positive cells in all women (p<0.01), but there was no increased production seen in pregnancy compared to non pregnant controls. The CRTH2 agonists did not cause an increase in IL-4 production in CRTH2 positive cells, but surprisingly led to a reduction in CRTH2 expression. The mean fluorescence intensity of CRTH2 expression reduced from 86.3 to 64.3 with 15dPGJ2 (p=0.015) and to 55.2 with Pyl A (p<0.001)

The increase in CRTH2 expression in pregnancy is consistent with the T helper 2 bias of pregnancy. The reduction in expression seen in preterm labour could reflect a switch in the bias to T helper 1 cells in the presence of inflammation/ infection. The functional role of CRTH2 was measured by IL-4 production which was not increased in pregnancy and cannot be modulated by the use of CRTH2 agonists. The down-regulation of CRTH2 expression with agonist treatment may represent a negative feedback mechanism for an unidentified functional role of CRTH2 in pregnancy.

T-239

In Utero Exposure to Estriol Selectively Alters Uterine Gene Expression.

Yuping Zhou, Sirisha Gudavalli, Geraldine Brichant, Hugh Taylor. *Obstetrics & Gynecology, Yale University, New Haven, CT, USA.*

Introduction: Estrogens are critical for the development of female reproductive organs and adult function. Estradiol (E2) is the predominant estrogen during reproductive years, while Estriol (E3) is produced in significant amount only during pregnancy. It is unclear if E3 has any function in pregnancy. An effect of E3 on development of the uterus and subsequent adult E2 responsiveness has not previously been investigated.

Methods: 8 pregnant CD-1 mice were continuously treated with E3 (200ug/kg/day) or vehicle control via osmotic minipump beginning on day 9 of gestation for 7 days. At 6 weeks, the female offspring (12/group) were oophorectomized; after 2 weeks they were treated with a single IP injection of either 300 ng estradiol (E2) or vehicle. 6 hours after injection the uteri were removed and RNA isolated. Total RNA was labeled and hybridized to a mouse BeadChip WG-8 expression microarray (Illumina). Data was normalized, and genes showing significant positive or negative change (more than 2-fold) versus control were identified, and verified by real time RT-PCR.

Results: Prenatal exposure to E3 had a significant impact on uterine global gene expression profile. As adults a total of 99 genes demonstrated a permanently altered expression pattern (56 upregulated and 43 downregulated) in the in utero E3-treated offspring compared to vehicle-treated controls. For example, adiponectin, an adipokine related to menstrual function, was upregulated in the offspring of E3-treated females.

An additional set of genes showed altered response only in response to E2 stimulation; expression of 59 genes (14 up and 45 down) was altered in response to E2 stimulation in E3-treated offspring. For example, Muc1 was down-regulated in E2 –treated offspring who were exposed to E3 in utero.

Among all altered genes (both the E3 and E3-E2 groups), 18 genes were involved in lipid metabolism; 15 genes in hormone metabolism/signaling; 18 genes in immune signaling.

Conclusions: Prenatal exposure of mice to E3 permanently changes the global gene expression profile and estrogen responsiveness of the uterus. A number of genes involved in reproduction are developmentally programmed by E3. E3 likely plays a role in developmental programming of estrogen response in the uterus.

T-240

Progesterone Withdrawal in the Pregnant Guinea Pig Is Mediated by Decreased Uterine PR Expression and Stimulated by Prostaglandins *In Vivo*. Toni Welsh,[1] Steve Caritis,[2] Jonathan Hirst,[1] Sam Mesiano,[3] Tamas Zakar.[1] *[1]Mothers and Babies Research Centre, University of Newcastle, Australia; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA; [3]Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH.*

Parturition in the guinea pig is not accompanied by changes in circulating progesterone levels and can not be delayed by exogenous progestin administration. A functional progesterone withdrawal is thought to occur in this species at the level of the target tissue, the myometrium. The purpose of this study was to determine whether progesterone withdrawal in the pregnant guinea pig is mediated by changes in uterine progesterone receptor (PR) expression. We have previously reported that prostaglandins (PGs) alter PR expression in a human myometrial cell line, and we therefore also investigated whether PGs regulate uterine PR expression in pregnant guinea pigs. Uterine tissue (myo-endometrium) was collected from pregnant dams at six time points between 45 days gestation and term labor (65-70d). A PGE_2 analog, sulprostone (0.25 mg), or vehicle were administered to pregnant animals at 45d and tissues were collected 16hr later; this treatment protocol induces labor within 24hr in the guinea pig. The PGHS inhibitor piroxicam (5 mg/kg/day) or vehicle were administered from 55d until term; we have previously reported that piroxicam delays labor onset in guinea pigs. PR-A and -B protein levels were measured by immunoblotting. Uterine PR-A and -B protein content decreased significantly throughout the last third of gestation (P<0.05, ANOVA; >85% decrease between 45d and labor), indicating that functional progesterone withdrawal in the pregnant guinea pig is mediated by decreased uterine PR expression. Uterine PR-A levels were significantly higher in animals treated with piroxicam compared to vehicle-treated controls (P=0.032; t-test), and were significantly lower in sulprostone-treated animals compared to controls (P=0.0168). PGs therefore stimulate progesterone withdrawal in the pregnant uterus and inhibition of endogenous PG synthesis prevents the normal decrease in uterine PR expression that precedes labor onset. We speculate that, in addition to their acute roles in stimulating myometrial contraction, fetal membrane rupture and cervical softening, intrauterine PGs stimulate progesterone withdrawal in the myometrium and play a key role in initiating the full parturition cascade.

T-241

PGDH Expression Decreases at Term before Labor Onset in Guinea Pig Fetal Membranes. Toni Welsh,[1] Jonathan Paul,[1] Hannah Palliser,[1] Jonathan Hirst,[1] Sam Mesiano,[2] Tamas Zakar.[1] *[1]Mothers and Babies Research Centre, University of Newcastle, Newcastle, Australia; [2]Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH.*

Prostaglandins (PGs) are important paracrine mediators of parturition, and as such, are key targets for the development of interventions that aim to arrest preterm labor and prevent preterm birth. The mechanisms that regulate intrauterine PG concentrations during pregnancy are largely unknown, however. The purpose of this study was to determine intrauterine expression of the chief PG inactivating enzyme, 15-hydroxy-PG dehydrogenase (PGDH), with advancing gestation and labor onset in the guinea pig, an animal model in which the endocrine control of pregnancy and parturition is analogous to that of women. Intrauterine tissues [amnion, visceral yolk sac (VYS), placenta and myo-endometrium] were collected at six time points between 45 days gestation and term labor (65-70d). PGDH mRNA abundance was measured by real-time RT-PCR and PGDH protein levels were measured by immunoblotting and localized by immunohistochemistry. The highest relative abundance of PGDH mRNA and protein was measured in the VYS, an equivalent membrane to the chorion. PGDH mRNA abundance decreased significantly in the VYS and amnion throughout late pregnancy (P < 0.05, nested ANOVA with Tukey's multiple comparison test). PGDH protein was robustly expressed in the VYS mesoderm and the nadir of VYS PGDH protein abundance was measured at term before labor onset. There was a significant positive correlation between VYS PGDH mRNA and protein levels (Spearman's rank correlation, P < 0.0001). PGDH mRNA and protein levels in placenta and myo-endometrium varied throughout late pregnancy, without a trend to increase or decrease with advancing gestation. PGDH protein in the placenta was concentrated in the parietal yolk sac (PYS) lining the placental surface and in placental blood vessels. We observed strong expression of PGDH protein in endometrium with virtually no expression in myometrium. These data indicate that the VYS, PYS and endometrium prevent the transfer of biologically active PGs to the myometrium for most of pregnancy. Decreased PGDH expression in the fetal membranes at term may allow for decreased PG metabolism and increased PG transfer to the uterus in order to stimulate labor onset. The guinea pig is an excellent model in which to further our understanding of the role and regulation of intrauterine PGs in pregnancy and parturition.

T-242

Prostagladin (PG) F2α Regulates Output of Interleukin (IL)-6, IL-8, MCP-1 and VEGF in Pimary Human Myometrial Smooth Muscle Cells (HMSMC). Chen Xu,[1,2] Xin Fang,[2] Stephen Wood,[3] Donna M Slater,[3,4] Xin Ni,[1] David M Olson.[2] *[1]Physiology, Second Military Medical University, Shanghai, China; [2]OB/GY Pediatrics and Physiology, University of Alberta, Edmonton, AB, Canada; [3]OB/GY, University of Calgary, Calgary, AB, Canada; [4]Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada.*

Background: PGF2α, as the potent uterine contractile agonist during labor, may have additional roles in converting the uterus of pregnancy into delivery. Mounting evidences support the interactions between PGF2α and cytokines which involved in luteolysis and other related immune reactions during pregnancy. Further, the initiation of labor showed a close correlation with the infiltration of leukocytes into human myometrium and cervix and the formation of a pro-inflammatory environment. The intriguing possibility is that PGF2α may be involved in regulating the labor-associated inflammatory reaction.

Hypothesis: PGF2α regulates output of pro-inflammatory cytokines in HMSMC.

Methods: Term-non-labor, primary HMSMC were cultured from lower segment (LS) and upper segment (US) biopsies. After 24h treatment with PGF2α (0.01-10μM), the medium supernatants were collected for Multiplex analysis followed by ELISA mass determination for specific cytokine output.

Results: Multiplex analysis indicated that 20 of 42 cytokines were up-regulated with PGF2α stimulation (the remainder showed no change or undetectable). IL-6 and vascular endothelial growth factor-165 (VEGF) output were 9- and 3-fold higher ($p<0.01$), respectively, in LS than in US at basal (no PGF2α) treatment. PGF2α significantly increased IL-6 with 5-fold ($p<0.01$) in the LS, but without marked effect in the US. VEGF output in both regions was stimulated 2-3 fold by PGF2α ($p<0.01$). Despite the similar basal outputs of IL-8 and MCP-1 in LS and US, they were both significantly stimulated by PGF2a 2- and 4-fold ($p<0.01$).

Conclusion and Significance: Term delivery has been well described as an inflammatory process. Our novel results indicate that PGF2α may assist the uterus in preparing for delivery through up-regulating the pro-inflammatory cytokines, such as IL-6, stimulating the chemotactic peptides, IL-8 and MCP-1, and possibly facilitating leukocyte recruitment to the uterus by increasing VEGF, which may increase vascular endothelial permeability. These actions may impact the recruitment of leukocytes to the uterus, release of inflammatory mediators and prepare the uterus for labor and delivery. NSFC, CIHR, & AIHS PreHot.

T-243

Global Expression Profile in Uterine Tissue of IL-6-/- and IL6+/+ Mice at Different Stages of Gestation and Postpartum. Youli Yao,[1,4] Xin Fang,[2] Sarah A Robertson,[3] J Filkowski,[1] Gerlinde A Metz,[4] Igor Kovalchuk,[1] David M Olson.[2] *[1]Biological Sciences, U of Lethbridge, Lethbridge, AB, Canada; [2]OG/GYN, Pediatrics, Physiology, U of Alberta, Edmonton, AB, Canada; [3]OB/GYN, U of Adelaide, Edmonton, SA, Australia; [4]Neuroscience, U of Lethbridge, Lethbridge, AB, Canada.*

Introduction. Interleukin-6 (IL6) abundance in amniotic fluid and uterine tissues increases in late gestation or with infection-associated preterm labor. IL6 has a key role in controlling the progression of events culminating in parturition. It acts downstream of luteolysis in the uterus to regulate genes involved in the prostaglandin-mediated uterine activation cascade. IL6-/- (null) mutant mice deliver 24h later than wild-type mice, despite no change from the normal decrease in circulating progesterone concentration following luteolysis.

Objective. Analyze gene expression in IL6-/- (null) and IL6+/+ WT mouse uterus during gestation, parturition and postpartum.

Methods. Animals were sacrificed at gestational day (GD)19.5, delivery, or 24h postpartum and uteri removed. N=3-4 per sampling group. RNA was extracted

and subjected to Illumina Mouse v2.0 Expression BeadChips and the gene expression analysis was performed using Illumina iScan.
Results. Each GD group formed a separate gene cluster. Common and unique gene groups were found to be significantly and differentially up or down regulated. Interestingly, IL6 null dams showed specific patterns in down or up regulated groups of genes at GD19.5, delivery and postpartum stages (Fig. 3).
Discussion. Genes commonly or differentially regulated at different gestation stages in IL6-/- (null) and IL6+/+ WT mouse uteri were identified. Further analysis will identify gene candidates associated with delayed parturition in null animals and IL6 pathways involved. Gene expression analysis will be confirmed via qRT-PCR and Western blot approaches.
Acknowledgements. Australian NHMRC, AIHS ITG Preterm Birth and Healthy Outcomes Team, University of Lethbridge Illumina Facilities.

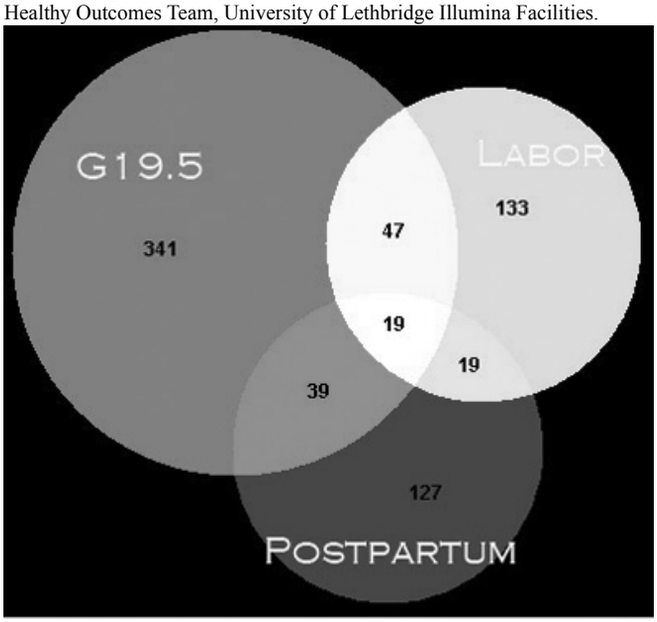


Fig 3. Genes significant differentially changed between IL6 null and WT mice at different gestation stages

T-244

Uterine Progesterone Receptor B (PRB) Promotor DNA Is Methylated in Delivering Mice. Youli Yao,[1] Xin Fang,[2] Nardhy Gomez-Lopez,[2] Sarah A Robertson,[3] Shlomit Goldman,[4] Eliezer Shalev,[4] Igor Kovalchuk,[1] Gerlinde A Metz,[5] David M Olson.[2] *[1]Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada; [2]OB/GYN, Pediatrics, Physiology, University of Alberta, Edmonton, AB, Canada; [3]OB/GYN, University of Adelaide, Adelaide, SA, Australia; [4]Rappaport Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel; [5]Canadian Centre for Behavioural Neurosciences, University of Lethbridge, Lethbridge, AB, Canada.*
Introduction. Through specific receptors in uterine tissues, progesterone is a key player in the process and maintenance of pregnancy. Current thinking suggests that in the rat the PR isoform B (PRB) is dominant over PRA before contractions but the PRA/PRB expression ratio decreases at term to allow delivery [Fang et al., 2002]. Through a methylation-specific PCR approach it was recently found that the PRB promoter is selectively methylated in human decidua at delivery at term (Shalev & Goldman - unpublished). Methylation effectively prevents promoter function and gene transcription. PRB methylation in rodents is unstudied.
Objective. To determine whether the PRB promoter is methylated in mice in late gestation and/or at delivery.
Methods. C57BL/6 mice were killed at gestational day (GD) 15, 19.5 and in labour and the uteri excised and frozen. We used a bisulfite conversion and a sequencing approach that targeted the PRB promoter and gene body regions.
Results. We discovered that the uterus of the laboured mice had several CpG islands (-229bp) that were up to 50% methylated. No methylation was observed in the same region at GD15 and GD19.5 mice. Meanwhile, the CpG islands within the PRB gene body and within PRA promoter remained unmethylated (1 to +281bp relative to PRB starting codon, which corresponds to PRA promoter region).
Discussion and Conclusion. Although circulating progesterone levels fall dramatically prior to delivery in mice, these results suggest that the methylation status of the PRB promoter plays an important role in the PR expression at delivery in mice, perhaps by decreasing PR responsiveness to residual progesterone.
Reference. X Fang et al. Am J Physiol Endocrinol Metab 283:1167-72, 2002.
Acknowledgement. AIHS ITG Preterm Birth and Healthy Outcomes Team, CIHR

T-245

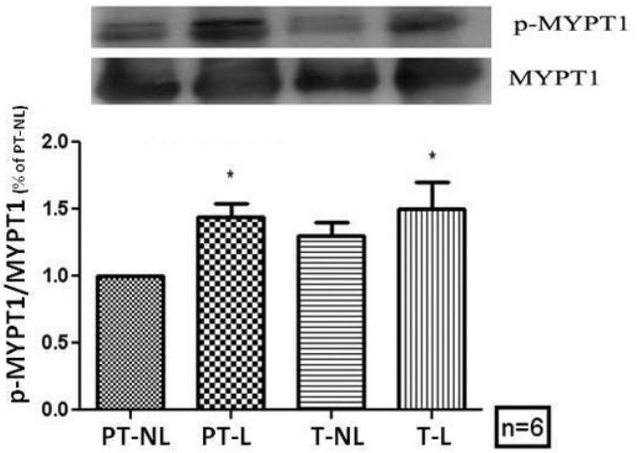
Withdrawn by Author

T-246

Integrin Localization to Focal Adhesion Sites in Pregnant Human Myometrium. Heather R Burkin,[1] Cherie A Singer,[1] Iain LO Buxton.[1,2] *[1]Pharmacology, University of Nevada School of Medicine, Reno, NV, USA; [2]Obstetrics and Gynecology, University of Nevada School of Medicine.*
Focal adhesions are integrin rich membrane microdomains that structurally link the cytoskeleton to the extracellular matrix and transmit mechanical signals. In the pregnant uterus increases in integrin receptor expression and activation are thought to be critical for focal adhesion development and formation of a mechanical syncytium required for labor. We performed experiments to determine which integrins are upregulated and localized to focal adhesions in the pregnant human myometrium. We used real time qPCR microarray analysis to detect significant increases at the transcript level for integrins A1, A3, A5, A7, AV, B1, B2, B3, and B5 in both nonlaboring and laboring pregnant human uterine myometrium compared to nonpregnant myometrium. At the protein level ITGA5, ITGA7, ITGAV, and ITGB3 were significantly increased in pregnant laboring and non-laboring human uterus by semiquantitative western blotting. We did not observe significant pregnancy-induced changes in ITGA1, ITGA3, or ITGB5 at the protein level. Finally confocal microscopy was used to colocalize ITGA5, ITGA7, and ITGB1 with focal adhesion proteins in the term human uterus. These data are consistent with the localization of integrins a5b1 and a7b1 at focal adhesions where they may mediate stretch-induced signaling during pregnancy and labor.

T-247

Chronic Inhibition of the RhoA/ROCK Pathway Is Not Involved in the Maintenance of Myometrial Quiescence during Pregnancy. Jorge A Carvajal, Liliana R Garmendia, Ana M Delpiano, Jose A Poblete, Mauricio A Cuello. *Obstetricia y Ginecologia, Pontificia Universidad Católica de Chile, Santiago, RM, Chile.*
Introduction: The mechanisms underlying pregnancy myometrial quiescence are poorly understood. We proposed that the chronic inhibition of the myometrial RhoA/ROCK pathway plays a main role in the maintenance of the myometrial quiescence. We also postulate that RhoA/ROCK inhibition is mediated by over-expression of Rnd proteins. **Materials and Methods:** Myometrium was obtained at the time of cesarean sections from four groups of pregnant women: preterm not in labor (PT-NL), preterm in labor (PT-L), term not in labor (T-NL) and term in labor (T-L). (preterm 30-34 weeks; term 38-41 weeks). mRNA and protein levels of RhoA, ROCK (I and II) and Rnd (1, 2, 3) were evaluated by semi-quantitative RT-PCR (n=6) and Western Blot (n=8). ROCK activity was studied by a substrate phosphorylation assay (MYPT1). **Results:** mRNAs of RHOA, ROCK I-II, Rnd1, Rnd2 and Rnd3 were identified in all groups, in similar levels. Only Rnd2 mRNA was significantly increased during quiescence (PT-NL). The studied proteins were present in all groups. Both RhoA protein and ROCK activity increased in labor samples, either preterm (PT-L) or term (T-L)



Discussion: RhoA/ROCK pathway (protein levels or activity) was not inhibited during quiescence. Rnd proteins were not increased during quiescence. These results do not support the role of the inhibition of RhoA/ROCK pathway in the maintenance of human myometrial quiescence. The increased RhoA/ROCK activity during labor suggests a role for this pathway in the process of cell contraction.

Financial Support: FONDECYT 1090616.

T-248

Determination of the Complete Transcriptome of Human Myometrial Smooth Muscle at Term by Laser Capture Microdissection (LCM) and RNA-seq. Yi-Wah Chan, Andrew M Blanks. *Reproductive Health, Warwick Medical School, United Kingdom.*

Introduction

It is well appreciated that tissues in complex organisms differentiate from a common progenitor by epigenetic modification of the genome. The differentially modified genomes give rise to cell specific transcriptomes, which in turn encode the proteins that determine phenotype and physiology. It is now possible to assess the full transcriptome of differentiated cell types within their natural environment by a combination of LCM and RNA-seq.

Hypothesis

RNA-seq and LCM would give unprecedented information about the allele specific expression of late pregnant myometrial smooth muscle cells (MSMC).

Methods

Frozen 8μm sections of myometrial(Term NIL, n=4)tissue were stained with cresyl violet and LCM of MSMC was performed. Extracted RNA quality was assessed via RIN scores on an Agilent Bioanalyser. Samples were selected based on a threshold of a RIN>6. Post-lasering stained sections were selected as a controls. cDNA was generated using the NuGEN Ovation RNA-Seq System.Reads were sequenced on an Illumina GAIIx. Transcriptome maps were generated using Bowtie (short read aligner) and Tophat (splice junction mapper). Differential expression and transcript abundance was assessed using Cufflinks.

Results

Typically, ~1ng/μl RNA was obtained per sample. 7 million paired end reads were obtained per sample. Differential expression analysis using Cufflinks detected ~24k sequences which had non-zero expression in all samples of which ~4k have no database reference. Hits with the highest FPKM (fragments per kilobase of exon model per million mapped fragments), a measure of transcript abundance, were tropomyosin 1, tropomyosin 2, myosin (heavy chain 11) and caldesmon 1. These MSMC markers support the success of isolating MSMC's. We compared transcripts generated by RNA-seq with our qRT-PCR data of all known K+ channels in the human genome. RNA-Seq was slightly less sensitive than qRTPCR but sequences detected by qRTPCR only could not be demonstrated at the protein level. Analysis of known key genes in parturition showed some novel information e.g. the KCNMA1 (BKca) expressed in MSMCs is a splice variant lacking exon 21.

Conclusions:

We have demonstrated that using RNAseq and LCM it is possible to obtain cell-type, allele, and sequence specific transcriptomic information. This technique has great power for all areas of reproductive medicine and will be particularly valuable when linking genomic information with physiology and disease.

T-249

Characterization of Hsf1 Expression in the Rat Myometrium. Sarah E Dinn, Daniel J MacPhee. *Biomedical Sciences, Memorial University, St. John's, NL, Canada.*

During pregnancy, the uterine smooth muscle, or myometrium, undergoes a series of developmental changes to ensure the uterus is prepared for the powerful contractions and timely delivery of the fetus, known as labor. In the rat myometrium, four phases of differentiation occur throughout the 23 day gestational period: proliferative, synthetic, contractile and labor phases. Heat shock transcription factor 1 (Hsf1) protein is present in the endometrium of the uterus; however, it is yet to be studied in uterine smooth muscle, the myometrium. Since heat shock factors are activated during cell differentiation and stress to upregulate the expression of key genes such as heat shock proteins (Hsps), it was hypothesized that Hsf1 would be highly expressed during specific phases of myometrial programming. Using immunoblot analysis, Hsf1 protein expression was found to be significantly elevated in the proliferative phase at day (d) 6 compared to d15, d17, and d22 of rat pregnancy ($p<0.05$). Preliminary immunoblot analysis of HSF2, another heat shock factor, also shows elevated expression during the proliferative phase. Immunofluorescence analysis demonstrated that Hsf1 was readily detectable in the myometrium in situ throughout gestation and was localized mainly in the cytoplasm of myometrial cells, with some granular staining in the nucleus. Hsf1 was readily detected in the longitudinal muscle layer and stayed at a fairly constant detection level throughout gestation. Hsf1 was also detected in the circular muscle layer throughout gestation, however detection decreased from d17- d21. Immunoblot analysis of pSer-230-Hsf1 protein expression, an indication of Hsf1 transcriptional activation, showed a significant increase early in gestation at d12 and then a significant decrease in expression between d12 and d22 ($p<0.05$). Based on the expression patterns observed, Hsf1 may play an important role to induce sufficient expression of Hsps and other developmental key genes necessary for myometrial programming during gestation.

T-250

Ligand-Independent Proliferative Action of the Androgen Receptor in Uterine Myometrial Cells – A Key Regulator for Myometrium Phenotype Programming. Xuesen Dong,[1] Liangliang Liu,[1] Ning Xie,[2] John Challis,[2] Stephen Lye.[2] *[1]Vancouver Prostate Centre, Dept. of Urologic Sciences, University of British Columbia; [2]SLRI, Dept. of Physiol. & Ob/Gy, University of Toronto.*

During pregnancy, myometrium undergoes phenotype programming starting from an early proliferative phase, an intermediate synthetic phase till a late contractile phase in which the cells are committed to labour. Thus phenotype changes in the early stage of pregnancy will affect the pregnancy progression and the timing of parturition. Steroid receptors play important roles controlling the proceedings of pregnancy. Previously we showed that the androgen receptor (AR) is at high levels during proliferative and synthetic phases, but dramatically decreased in the late contractile phase. The aim of study is to characterize the molecular function of AR in myometrial cells.

Using human myometrial cell line hTERT-HM as a model, we showed neither AR agonist (R1881) nor antagonist (MDV3100) impacts on myocyte proliferation. However, AR silencing resulted in a 50% decrease in cell growth rates (an action which is opposite to that in prostate cancer LNCaP cells where AR ligand-dependently regulates cell proliferation). FACs analysis confirmed AR knockdown induced a delay in G1-S phase transition of cell cycle. Synchronized hTERT-HM cells by serum-starve were added with FBS for 0-24hrs. In cells with scramble shRNA, G0/G1 population dropped from 95% to 78% at 18h, 40% at 21h and stayed at 50% at 24h. In cells with shAR, G0/G1 population maintained at 95-90% between 0-18h, started to drop only to 70% at 21h and kept at 70% at 24h. Concurrently, AR knockdown induced 80% decrease in cyclin D, A and B but not cyclin E levels, but a 10-fold increase in the level of p27, an inhibitor to cell cycle progression at G1 phase. AR silencing did not alter myometrial cell expression of IGF-1, but decreased by 50% IGF-1R protein expression. Downstream IGF-1R signalling pathways were also attenuated including PI3K/Akt/mTOR and JNK/MAPK pathways. Taken together, we conclude that AR (acting in a ligand-independent manner) is a key regulator of myometrial phenotype programming during early pregnancy. Decreased myometrial AR levels may result in reduced myocyte proliferative in early pregnancy and thus reduce the total pool of myocytes able to undergo hypertrophy during the synthetic phase. Together these events would reduce uterine growth and possibly predispose pregnancies to stretch-induced preterm labour.

Thursday

T-251

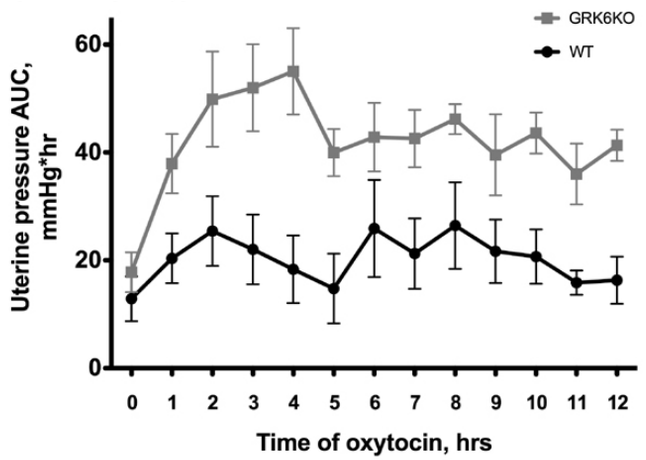
G Protein-Coupled Receptor Kinase 6 (GRK6) Knock-Out Mice Exhibit Enhanced Uterine Contractility in Response to Oxytocin Infusion. Chad Grotegut,[1] Lan Mao,[2] Amy Murtha,[1] Howard Rockman.[2] *[1]Obstetrics and Gynecology, Duke University, Durham, NC, USA; [2]Medicine and Cell Biology, Duke University, Durham, NC, USA.*

Objective: GRK6 phosphorylation of the oxytocin receptor (OXTR) following agonist activation allows for β-arrestin recruitment, which promotes OXTR desensitization. GRK6 knock-out (GRK6KO) mice exhibit a reproductive phenotype with increased incidence of appropriately grown, term stillborn pups. We hypothesize that enhanced uterine contractions in GRK6KO mice secondary to diminished OXTR desensitization produce this phenotype. We thus sought to determine the role of GRK6 in mediating oxytocin-induced uterine contractions in live mice receiving an oxytocin infusion.

Methods: Non-pregnant GRK6KO mice (n=5) and wildtype (WT) mice (n=5) underwent surgical implantation of an ambulatory uterine telemetry device (DSI PA-C10) that measures changes in uterine pressure and simultaneous placement of an osmotic pump delivering oxytocin at a rate of 5 Units/day. The cages holding these mice were placed over a receiver and changes in uterine pressure with time were recorded for 24 hours during oxytocin treatment. The area under the contraction curve was determined at one-hour intervals during the 24-hour treatment and compared between the two mouse types using two-way ANOVA.

Results: All mice exhibited uterine contrations in response to oxytocin treatment. GRK6KO mice exhibited enhanced uterine contraction response as measured by area under the contraction curve (AUC) compared to WT mice during 24-hour oxytocin treatment (Figure, p=0.012 for differences in curves by mouse genotype). Furthermore, the total area under the contraction curve during the 24-hour period was greater in the GRK6KO mice compared to the WT mice (p=0.019).

Conclusions: GRK6KO mice exhibit enhanced uterine contraction responses with oxytocin stimulation compared to WT mice. Diminished OXTR desensitization in GRK6KO mice may be producing this observed phenotype in non-pregnant mice. Further work in pregnant mice will determine whether diminished OXTR desensitization in GRK6KO mice is responsible for its reproductive phenotype of stillbirth.



T-252

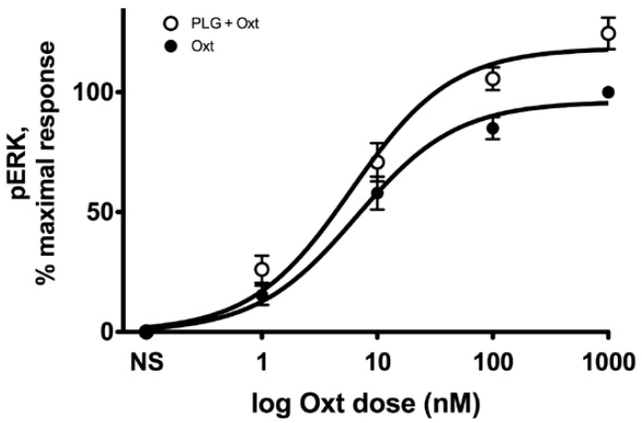
MSH-Releasing Inhibitor Factor (pro-leu-gly-NH2) Acts as an Allosteric Activator for Oxytocin-Induced MAPK Signaling but Does Not Affect Uterine Contractility. Chad Grotegut,[1] Liping Feng,[1] Amy Murtha,[1] Howard Rockman.[2] *[1]Obstetrics and Gynecology, Duke University, Durham, NC, USA; [2]Medicine and Cell Biology, Duke University, Durham, NC, USA.*

Objective: MSH-releasing inhibitor factor (pro-leu-gly-NH2, PLG) is a neuropeptide and an oxytocin fragment, containing the last three amino acids of the hormone oxytocin. We sought to determine if PLG affects OXTR-mediated MAPK signaling and uterine contractility.

Methods: HEK-293 cells expressing the OXTR were stimulated with oxytocin at concentrations of 1nM to 1μM, with or without pretreatment with PLG (10μM). MAPK activation was measured with western blot for phospho-ERK following oxytocin stimulation for five minutes at each concentration. To determine if PLG increased oxytocin-induced uterine contractions, uterine muscle strips from non-pregnant wildtype mice were suspended in a tissue organ bath and stimulated with oxytocin with or without pretreatment with PLG and the contraction responses measured. Dose response curves were constructed for each (MAPK activation and uterine contraction response) and compared using two-way repeated measures ANOVA.

Results: Pretreatment of HEK-293 cells expressing the OXTR with PLG increased oxytocin-induced phospho-ERK signaling compared to cells not receiving PLG pretreatment (Figure). PLG pretreatment led to 118% of maximal phospho-ERK activation by oxytocin alone (p=0.005). The increase in phospho-ERK seen with PLG was reversed with the PKC inhibitor, Ro-31. Pretreatment of uterine muscle strips from non-pregnant wildtype mice did not affect oxytocin-induced uterine contractions. Stimulation of cells or uterine muscle strips with PLG alone did not activate MAPK signaling or induce uterine contractions.

Conclusions: The oxytocin fragment PLG acts as an allosteric activator for oxytocin-induced MAPK signaling that is reversed by a PKC inhibitor, but does not increase G-protein-mediated oxytocin-induced contractions. Further work is needed to understand the molecular conformations of the OXTR that produce these findings.



T-253

An Elevated Leptin Obese Mouse Model Demonstrates Uterine Fibrosis and Decreased Uterine Contractility in Response to Oxytocin. Ravindu Gunatilake, Jason Yeh, Liping Feng, Friederike Jayes, Phyllis Leppert, R Phillips Heine, Amy Murtha, Chad Grotegut. *Obstetrics and Gynecology, Duke University, Durham, NC, USA.*

Objective: The underlying mechanisms accounting for labor dysfunction in the setting of maternal obesity are largely unknown. In the liver, leptin has been implicated in producing a fibrotic response following injury. We sought to determine if an elevated leptin obese mouse model demonstrates uterine fibrosis and impaired contractility.

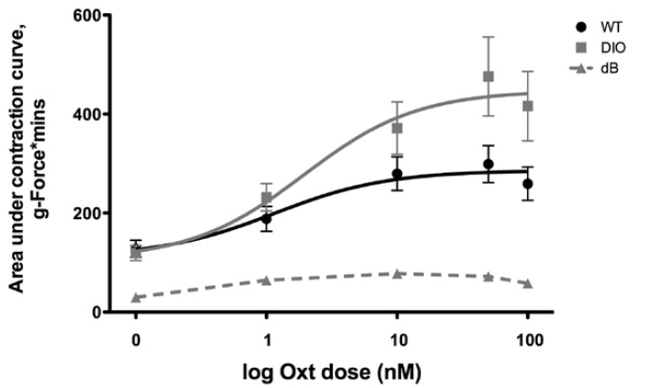
Methods: Uteri from non-pregnant wild type (WT) mice, normal leptin obese mice (diet-induced obese, DIO), and high leptin obese mice (db/db) were obtained at 12 to 16 weeks of age (n=10 for each group). One horn from each mouse was formalin-fixed, paraffin embedded, and stained with Masson's Trichrome. Digital whole-slide images were obtained and the percent of collagen staining relative to the entire uterine area was determined for each sample, reported as percent fibrosis, and compared using ANOVA. The second uterine horn was suspended in a tissue organ bath, treated with successively increasing oxytocin (1nM, 10nM, 50nM, 100nM) and the area under the contraction curve determined. The mean best-fit maximal area under the contraction curve was determined using non-linear regression and compared using two-way, repeated measures ANOVA.

Results:

The mean (SD) weight (g) for the WT, DIO, and db/db mice were 21.6 (1.1), 32.8 (5.0), and 49.1 (1.4), respectively (p<0.001). The mean (SEM) percent uterine fibrosis was greater in db/db mice (0.34 ± 0.07) compared to WT (0.07 ± 0.01) and DIO (0.07 ± 0.02) mice (p=<0.001). The mean (mean ± SEM) best-fit maximal contraction response (area under contraction curve, g Force*mins) to oxytocin was also significantly different among db/db (72 ± 6), WT (286 ± 21), and DIO (448 ±41) mice (p=<0.001, Figure).

Conclusions:

A high leptin obese mouse model demonstrates increased uterine fibrosis and decreased uterine contractility compared to normal weight mice and normal leptin obese mice. Whether this observed phenotype is a direct result of leptin action or due to other factors associated with obesity warrants further study.



T-254

A Sequential Gs/Gi Pathway Activation Is Required To Induce B3-Adrenoceptor (B3-AR) Mediated Human Myometrial Cell Proliferation In Vitro. T Hadi,[1] M Barrichon,[1] F Lirussi,[1] P Moutialon,[2] F Goirand,[1] M Dumas,[1] P Sagot,[2] M Bardou.[1] *[1]CIC-P803, INSERM; [2]Obstetrics & Gynecology, CHU, Dijon.*

BACKGROUND: In the current need to find safe and efficient tocolytic drugs, our team has focused on the interest of B3-AR stimulation for the treatment of preterm labor. We have previously shown that B3-AR stimulation by the selective agonist SAR150640 (SAR) induces myometrial cells (MC) proliferation in a Gs protein-dependant manner, and may thus lead to an inhibition of labor onset. The B3-AR is not internalized upon prolonged stimulation, but nothing is known about its potential negative regulation by a switch from Gs to Gi protein coupling, as it has been documented in the B2-AR.
OBJECTIVE: We assessed the effect of a sustained B3-AR stimulation on a potential Gs/Gi coupling switch and its impact on the B3-AR proliferative effects.
METHODS: Primary MC lines were established from myometrial biopsies obtained from women undergoing elective caesarean delivery for uncomplicated pregnancies before labor onset (written informed consent obtained, ethics committee approval). After 24h adhesion and 72h starvation, cells were treated with SAR 100nM (for 3', 8 and 48h). Proliferation was assessed by flow cytometry. Erk1/2 and Akt activation were assessed by western blotting. G-protein pathways involved were assessed using pharmacological agents: Melittin (100nM) and pertussis toxin (PTX, 200ng/ml) to inhibit Gs and Gi respectively, and U0126 (5µM) and Triciribin (10µM) to inhibit Gs and Gi respective targets: Erk1/2 and Akt.
RESULTS: SAR induced a 2.9* and 1.6*folds increase in Erk1/2 phosphorylation after 3' and 8h stimulation respectively, and a 2.03fold* increase in Akt phosphorylation after 48h of stimulation (*P<0.05 vs control). All activations were fully antagonized by melittin co-stimulation, while PTX only failed to antagonize Erk1/2 activation at 3' (2.49 fold increase vs PTX alone, P<0.05). Finally, 48h SAR stimulation induced a 21%* increase in cell number vs control (*P<0.05), an effect fully antagonized by U0126, melittin or PTX but only partially by triciribin (+15.41% cells only vs Triciribin alone, P<0.05).
CONCLUSION: This study shows that B3-AR stimulation in myometrial cells induces first a Gs and then a Gi pathway activation. Both pathways are required to lead to a full activation of Erk1/2 and a final Akt activation, suggesting that, instead of inhibiting B3-AR effect, the Gs/Gi switch potentiates its proliferative effect.

T-255

Characterization of Human Myometrial TREK-1 Potassium Channel Currents. Nathanael Heyman,[1] Scott Barnett,[1] Iain LO Buxton.[1,2] *[1]Pharmacology, University of Nevada School of Medicine, Reno, NV, USA; [2]Obstetrics and Gynecology, University of Nevada School of Medicine, Reno, NV, USA.*

TREK-1 is a stretch-activated, two-pore domain K+ channel (K2P) upregulated during gestation and present in low abundance in preterm laboring myometrium that we have hypothesized to play an important role in maintaining uterine quiescence during gestation by stabilizing the myometrial cell membrane potential. The precise mechanism that leads to the onset of labor is not fully known, however, it is believed that regulation of TREK-1 may play an important role in this process since hyperpolarization of the membrane is cnsistent with decreased calcium entry through voltage sensitive L-type calcium channels. Using whole cell voltage clamp techniques we were able to determine the presence of TREK-1 currents in both HEK293T cells overexpressing TREK-1 cloned from human myometrium, as well as in freshly isolated and telomerized pregnant human uterine smooth muscle cells. TREK-1 activation was accomplished by treatment with 10 µM arachidonic acid, a polyunsaturated fatty acid known to be elevated during pregnancy, as well as intracellular acidification (using $NaHCO_3$ and a pH 6.0 pipette solution). Treatment of cells with the nitric oxide donor GSNO (100 µM) activated the current consistent with a role for NO in uterine smooth muscle relaxation. Selective blockade of the TREK-1 current was achieved using the antagonist, fluphenazine, a piperazine antipsychotic. We conclude that TREK-1 currents are present in human uterine smooth muscle cells and their regulation by NO, arachidonic acid and pH is consistent with their participation in the regulation of gestinational quiescence.

T-256

Changes in Expression of PKA- and Acetylation-Related Signalling Molecules with Guinea Pig Pregnancy. M Karolczak-Bayatti,[1] N Bayatti,[1] NR Chapman,[2] BF Mitchell,[3] GN Europe-Finner,[1] MJ Taggart.[1] *[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; [2]Dept of Human Metabolism, Sheffield University, Sheffield, United Kingdom; [3]Dept of Obstetrics and Gynecology, University of Alberta, Edmonton, Canada.*

Mice and rat models of pregnancy and parturition are instrumental in improving our understanding of the cellular and tissue mechanisms of uterine activation accompanying labour. A marked difference between these rodents and humans, however, is that parturition in the former is preceded by a marked fall in maternal serum progesterone. Lately, attention has returned to considering the benefits offered by the guinea pig as an additional rodent model of human parturition given that, as in the human, withdrawal of maternal serum progesterone does not appear to be a pre-requisite for labor onset (Tong et al., this meeting). Recently, we have suggested that acetylation, in alliance with Protein Kinase A (PKA)-dependent signaling, is a key regulator of myometrial protein activity and function. Therefore, we explore the expression of key components of the PKA and acetylation pathways in guinea pig myometrium during pregnancy. Levels of the proteins involved in the pro-inflammatory cascade were also investigated.
Methods: Non-pregnant (NP) and late pregnant (P, d65-69) guinea pig uterine biopsies were homogenised and prepared for western blotting.
Results: We demonstrated no change in the expression of selected pro-quiescent myometrial proteins such as PKA subunits RIIα and CIIα in the tissue extracts from NP and P guinea pig myometrium. Class I lysine deacetylases (KDAC) alter the acetylation of nuclear and non-nuclear myometrial proteins. Levels of the non-nuclear residing KDAC8 decrease in myometrium of pregnant guinea pig compared to NP as did the nuclear-residing KDAC1 and 2. Conversely, the expressions of an important substrate for KDAC8, HSP20 and its putative nuclear acetyltransferase-CBP were increased in pregnancy. Regarding the NFκB pathway, higher levels of IκBα and Bcl3 were observed in P, whereas RelA was elevated in NP tissues.
Conclusion: These data highlight differences in the patterns of expression of proteins key to PKA/acetylation/inflammatory signaling pathways in guinea pig myometrium with pregnancy and point to the continued need for continued molecular characterisation of guine pig pregnancy as a comparator of human parturition.
Supported by MRC (UK, G0900525) & AIHS & WCHRI (Canada)

T-257

Uterine Myometrial Endoplasmic Reticulum Stress Response Determines Gestational Length. Chandrashekara N Kyathanahalli, Aravind Suresh, Jeyasuria Pancharatnam, Jennifer C Condon. *Obstetrics and Gynecology, Magee Women's Research Institute, Pittsburgh, PA, USA.*

Parturition involves coordinated contraction of the uterine myometrium. Uterine quiescence must therefore be tightly regulated to prevent preterm birth. Earlier, we provided convincing evidence in the pregnant mouse for the tocolytic role of caspase-3 (CASP3) in maintaining uterine quiescence through disabling of contractile architecture and subsequent upregulation in anti-apoptotic signal cascade to limit its proteolytic function in pregnant uterus.
We propose that pregnant uterus heralds an adaptive response at the level of endoplasmic reticulum [ER], which elicits unfolded protein response [UPR] that regulates uterine CASP3 activity. We questioned whether manipulation of uterine UPR-response using known inducers/inhibitors of ER stress alter the

tocolytic potential of CASP3 and cause either preterm birth or delay in labor. The pregnant mouse model was employed to examine gestational dependent changes in the expression of proteins involved in ER stress/ UPR-response and altered timing of parturition following administration of Tunicamycin [TM], a known inducer of ER stress. Expression of protein components of UPR signaling cascade (CASP12, CASP3, phospho-PERK, CHOP and BiP) were measured by western blotting of uterine myometrial samples from control pregnant mice and pregnant mice administered with TM at various dosage levels [0-2mg/kg bw, single, i.p] at E 15 and 16.

A distinct differential response in the expression of the UPR proteins was discernable in TM-administered pregnant mice. Expression of BiP, and the levels of active CASP12, CASP3 remained elevated at the dosages tested. Although, a low dose of TM [0.04mg/kg bw] did not induce preterm labor or any clinical symptoms of mortality, termination of pregnancy was observed at higher dosages [> 0.2 mg/kg bw; 24h post treatment]. Taken together, the preliminary data suggest that a tonic increase in ER stress upregulates UPR associated chaperone proteins which manage the apoptotic potential of CASP3; while excessive ER stress despite an increase in UPR fails to maintain uterine quiescence and leads to preterm birth. Currently we are employing small molecule inhibitors and chaperone proteins to further define whether activation of the UPR will prevent the onset of pre-term birth under conditions of chronic / excessive ER stress. These studies may reveal novel targets for preventive interventions.

T-258

Characterisation of the Small Heat Shock Proteins, HSP27 and αB-Crystallin in a Murine Model of Gestation and Inflammatory/Infection-Induced Pre-Term Labour. David A MacIntyre,[1] Syedah Ashraf,[1] Yun S Lee,[1] Bronwen Herbert,[1] Mark R Johnson,[2] Phillip R Bennett.[1] *[1]Surgery and Cancer, Imperial College London; [2]Obstetrics and Gynaecology, Chelsea and Westminster Hospital.*

Small Heat Shock Proteins (sHSPs) are implicated in the contractile machinery of smooth muscle cells where they regulate muscle tone and contractile activation. In non-contractile smooth muscle cells, sHSPs exist in large multimeric complexes that participate in protein folding and chaperoning. Human myometrial contraction is associated with a decrease of αB-crystallin and increased phosphorylation of HSP27, the latter of which facilitates its interaction with, and stabilisation of, pro-contractile fibrillar actin[1]. To further explore the role of these proteins in the context of pregnancy and labour, we investigated their expression in murine models of gestation and inflammatory/infection-induced pre-term labour (PTL). Myometrial samples from non-pregnant, gestational day 6 (E6), E11, E18 and during active labour were examined for concentration of total HSP27 and αB-crystallin using western blotting (n=4). HSP27 levels increased throughout gestation, peaking at E18 just prior to labour. In contrast, αB-crystallin decreased throughout gestation becoming almost non-detectable in labouring samples. For the PTL model, timed pregnant CD-1 mice at E16 were administered with an intrauterine injection of lipopolysaccharide (E. Coli: O111) or PBS control and myometrial samples collected at 1h, 2h, 4h and 6h post-injection and assessed for HSP27 and αB-crystallin levels (n=4). No change in total HSP27 concentrations were detected in response to LPS treatment however, αB-crystallin levels were decreased at 6h compared to PBS controls. These results indicate that both normal labour and inflammation/infection-induced PTL involve changes in the sHSPs. Future work is directed toward exploring the interactions between these proteins throughout gestation and parturition. We anticipate that elucidation of sHSP function will aid in the identification and future design of novel tocolytic interventions.

[1]MacIntyre DA., et al. Endocrinology 2008 149:245-252

T-259

Characterization of HspB8 Expression in Rat Myometrium. Noelle L Marsh, Angela L Wareham, Adam Green, Daniel J MacPhee. *BioMedical Sciences, Memorial University, St. John's, NL, Canada.*

The small heat shock proteins (sHsps; HspB1-11), form a ubiquitous family of molecular chaperones that enable tissues to adapt to changes in their local environments during differentiation or disease conditions. HspB8 has been shown to be highly expressed in smooth muscle, but the expression and function in the uterine smooth muscle or myometrium remains unknown. The goal of this project was to characterize the expression and potential regulation of HspB8 protein in pregnant rat myometrium during myometrial programming. Rat myometrium was collected from non-pregnant rats and pregnant animals at d6, d12, d15, d17, d19, d21, d22, d23 (labour) and 1 day post-partum (PP) and RNA and protein samples prepared for qPCR and immunoblot analysis, respectively. Using qRT-PCR it was determined that HspB8 mRNA expression was significantly ($p<0.05$) increased after d15. Immunoblot analysis determined that HspB8 protein expression was elevated at d15 and d17 compared to expression at NP and at d15 compared to d22, d23 and PP (One way ANOVA; n=5; $p<0.05$). The upregulation of HspB8 at mid-gestation coincides with the synthetic phase of myometrium differentiation. To determine the spatial localization of HspB8 protein throughout gestation, immunofluorescence analysis was conducted. Detection of HspB8 protein in the circular and longitudinal muscle layers revealed that it was primarily localized in the cytoplasm throughout gestation, particularly at mid-gestation; however, there was also detection of HspB8 near cell membranes as well as an absence of any nuclear staining. Recently, there is also evidence that HspB8 and the co-chaperone Bag-3 (Bcl-2-associated athanogene 3) form a complex which plays an important role in proteostasis and muscle maintenance. Preliminary immunoblot analysis indicates that Bag-3 expression is also upregulated in the synthetic phase of gestation similar to that of HspB8. This suggests that a HspB8-Bag-3 complex may be important for myometrial hypertrophy as this process would require regulation of protein quality control.

Overall, the patterns of expression of HspB8 in the myometrium indicate that it could have a role at mid-gestation as an anti-apoptotic protein when a caspase cascade is known to be initiated and/or associate with Bag3 and regulate protein quality control during hypertrophy. These data add to the body of knowledge indicating sHsps are important for myometrial programming during pregnancy.

T-260

Expression, Localization, and Function of Purkinje Cell Protein 4 (PCP4) in Human Myometrium. Clifford W Mason, Yafeng Dong, Hui Zhou, Carl P Weiner. *Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS, USA.*

Introduction: Purkinje cell protein 4 (PCP4, aka PEP19) is a small calmodulin-binding protein that binds both calcium-free and -bound calmodulin to regulate the calmodulin-mediated signal. In a comprehensive genomic analysis of myometrium from 4 groups of women consisting of term not in labor (TNL), preterm not in labor (PTNL), term in labor (TL), and preterm in labor (PTL), we determined the expression of this molecule is significantly decreased in PTL. There is little known of the regulation and function of PCP4 in myometrial smooth muscle cells (MSMC) during pregnancy and labor. We hypothesize that a decrease in PCP4 expression in MSMC leads to activation of Ca2+-CAM-mediated signal and MSMC contraction.

Objectives: Characterize PCP4 expression, localization, and function in human myometrium.

Study Design: PCP4 mRNA and protein was quantified in human myometrium from women at term and preterm with or without labor (n=6 subjects/group) using Q-rtPCR and Western blot. Double stained indirect immunofluorescence was used to determine the cell-specific localization of PCP4. Myometrial smooth muscle cells were identified with an anti-desmin antibody. Myometrial interstitial cells were identified by staining for de-phosphorylated connexin 43 and the sarcoplasmic reticulum by staining for the Ca2+-ATPase pump (SERCA). PCP4 was expressed in immortalized pregnant and non-pregnant human myometrial smooth muscle cell lines, which were initially void of PCP4 expression.

Results: PCP4 mRNA and protein were significantly decreased in myometrium from PTL compared to TL, TNL, and PTNL, confirming the microarray findings. PCP4 was found only at the periphery of smooth muscle bundles and localized primarily to the sarcoplasmic reticulum. Transfection of PCP4 into pregnant and non-pregnant human MSMC was confirmed by Q-rtPCR and Western blot analysis.

Conclusion: PCP4 is uniquely altered in association with spontaneous PTL. It co-localizes to the sarcoplasmic reticulum of MSMC suggesting PCP4 may regulate myometrial intracellular Ca2+ levels. Transfection of PCP4 into human MSMC will provide an in vitro model for future studies to better understand PCP4 function during pregnancy. These studies promise new insights into the role of PCP4 in the regulation of myometrial contraction and may lead to the design of new approaches for controlling myometrial activity during spontaneous preterm labor.

T-261

Kv2.1 Is the Major Component of Late Outward Potassium Currents Observed in Myometrium. Conor McCloskey, Elizabeth Bailey, Anatoly Shmygol, Andrew M Blanks. *Reproductive Health, Warwick Medical School, United Kingdom.*

Introduction:

K+ channels play a major role in controlling myometrial contractions and quiescence by modulating myometrial smooth muscle cell (MSMC) membrane potential. In a genomic screen of laser captured MSMC mRNA we have previously identified candidate genes that may be important at the functional level. Here we report that Kv2.1 is the major K+ conductance in MSMCs.

Hypothesis:

Kv2.1 is a major K+ channel in MSMCs.

Methods

Myometrial biopsies were taken in women at the time of C-Section (PTL,PTNIL,TNIL/TLAB, n=6) and mice GD15-18 (n=5/GD). Isolated MSMCs were perfused with Krebs TES solution at 37°C and studied using the amphotericin perforated patch clamp technique. In vitro force recordings before and after administration of the inhibitor, stromatoxin (ScTx) determined the functional contribution of Kv2.1, whilst membrane potential (Vm) was determined by administration of ScTx under current clamp conditions using microelectrodes.

Results

In voltage clamp experiments cells stepped from HP -60mv to potentials from -150 to +80mV for 500 ms evoked large outward currents very similar in kinetics to Kv2.1. These outward currents proved insensitive to both 10 nM margatoxin and 10 μM XE991, however 100 nM ScTx significantly blocked the outward currents (control current at +40 mV of 4.979 ± 0.616 pA/pF reduced to 2.197 ± 0.254 pA/pF). When fitted to a Boltzmann relation the conductance V1/2-act of this ScTx sensitive current was -7.96 ± 1.46 mV, indicative of Kv2.1.In current clamp experiments ScTx alone had minimal effects.When combined with Paxilline (1μM) and Apamin (100nM) a profound increase in action potential overshoot was apparent along with a prolonging of the action potential waveform indicating redundancy and repolarization reserve exist amongst myometrial potassium channels. Human MSMC demonstrated abundant full length, mRNA transcripts for KCNB1 (KV2.1) as determined by RNA-seq combined with LCM. This was confirmed at the protein level by western blotting with immunoreactive bands at the predicted molecular mass of 96KDa.

Conclusions:

Kv2.1 mediates the majority of late outward current in MSMCs. Significant repolarization reserve exists in the long myometrial action potential giving the contraction robustness and durability. This has implications for treatments targeted at K+ channels aimed at modulating contractions for therapeutic benefit as well as scientific studies aimed at understanding channel function.

T-262

Extracellular Acidosis Stimulates Uterine Contractions. Karen Noble, Susan Wray. *Physiology, University of Liverpool, Liverpool, Merseyside, United Kingdom.*

Introduction

Extracellular acidosis is emerging as an important player in physiological cell signaling**.** The effects of extracellular acidosis on the uterus may be critically important as It is widely accepted that during labor, the uterus undergoes transient ischemia and ischemia will induce both intracellular and to a greater degree, extracellular acidosis [1]. The aim of present work is to determine how extracellular acidosis affects uterine contractions in term pregnant and laboring mice.

Methods

Measurements of spontaneous contractions and in some experiments simultaneous measurements of intracellular pH (with SNARF) were made on strips (1X 4mm; 3mg) of longitudinal myometrium taken from term pregnant or laboring CD-1 mice. The extracellular pH (pH_e) of the strips was clamped by superfusing strips in a 2ml bath with HEPES buffered physiological solutions at control or acidic pH (pH 7.4 or 6.9 respectively). Intracellular pH (pH_i) was decreased by the isosmotic addition of sodium butyrate (20mM) to the bath. Data are mean ± s.e.m. and analyzed using t-test.

Results

Extracellular acidosis (pH 7.4 to 6.9 for 10 min), increased the amplitude and frequency of spontaneous myometrial contractions by 21.1 ± 2% (p=0.006) and 117 ± 16% (p= 0.012; n=7) respectively. A similar or possibly more potent effect was seen in laboring mouse myometrium, (n=2).

Extracellular acidosis decreased the pH_i of strips by 0.14 ± 0.04 pH units. Previous studies in rat and human myometrium have shown that intracellular acidosis inhibits myometrial activity. In the present study, butyrate (control pH_e 7.4 for 5 min) decreased pH_i by 0.14 ± 0.02 pH units (From pH 6.99 ± 0.02 to pH 6.85 ± 0.02; n=3) and completely inhibited spontaneous myometrial contractions.

Conclusions

Extracellular acidosis stimulates uterine contractions in the late pregnant mouse overcoming an intracellular acidosis which will inhibit myometrial activity. A similar effect has been reported in human myometrium [2]. This stimulation appears to be even more during labor. These data in term and successfully laboring murine tissues will help elucidate a novel signaling mechanism that may help maintain human uterine activity during the transient periods of ischemia that occur during labor.

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T-263

Bile Acids Upregulate the Oxytocin Receptor and Increase Contractility in Human Myometrium. Jordan E Read,[1] Sung H Kim,[1] Draga Tchipeva,[1] Catherine Williamson,[2] Phillip R Bennett,[1] Vasso Terzidou.[1] *[1]Parturition Research Group, IRDB, Imperial College, London; [2]Maternal and Fetal Disease Group, IRDB, Imperial College, London.*

Background: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy specific liver disease associated with a raised bile acid profile. Although prevalence varies geographically and cases differ in severity, preterm labour is common but the mechanism is unknown. One possible mechanism is via the effect of bile acids upon myometrial contractility.

Aim: To investigate the effect of bile acids on OTR expression in human myocytes and on myometrial contractility.

Methods: Primary myocyte cell cultures were established from patients undergoing elective caesarean section at term. Myocytes were treated with Cholic acid (CA:20μM), Taurocholic acid (TCA:200μM) or Glycocholic acid (GCA:200μM) over a time-course of 4-24 hours. RNA was extracted and QRT-PCR was performed for OTR, with results corrected for L-19. Protein was extracted and Western Blot analysis was carried out for OTR, with results corrected to β-actin. Smooth muscle strips were cut from myometrial biopsies obtained from patients undergoing elective caesarean section at term. Force, frequency and duration of spontaneous and oxytocin induced contractions were analysed following acute treatment with TCA (100/200μM), GCA (100/200μM) or CA (20/40μM) or 16h incubation in 20μM CA.

Results: Treatment of primary human myocytes with CA, TCA and GCA significantly increases OTR expression by 4 hours at both mRNA and protein level (n=4 patients, $P<0.05$). The effect of CA is maintained through to 10 hours (n=4 patients, $P<0.05$) and for TCA and GCA returns to basal level by 24 hours. A positive effect on oxytocin induced contractions was observed after acute application of bile acids on spontaneously contracting myometrial strips and 16h incubation of smooth muscle strips with 20μM CA caused a significant enhancement in OT induced contractions (n=4 patients, $P<0.05$).

Conclusion: Bile acids may contribute to the onset of preterm labour in women with ICP through upregulation of the OT/OTR system and thus increased contractility of myometrial smooth muscle.

T-264

A New Slow Releasing, H_2S Generating Compound, GYY4137 Relaxes Human and Rat Pregnant Myometrium. Hayley Robinson,[1] Theodor Burdyga,[2] Susan Wray.[3] *[1]Physiology, University of Liverpool, United Kingdom; [2]physiology, University of Liverpool, United Kingdom; [3]Physiology, University of Liverpool, United Kingdom.*

There is a pressing need to increase understanding of how uterine contractility is controlled and to develop better tocolytics to reduce the morbidity and mortality associated with pre-term delivery. Thus a newly discovered endogenous molecule that may reduce contractility is of interest. It has been shown that the uterus possesses the enzymes to produce hydrogen sulfide (H_2S) from L-cysteine, namely cystathionine β-synthase and cystathionine γ-lyase. Reports have shown H_2S to be able to reduce contractions of rat and human myometrium making it an attractive target for clinical manipulation. However it is not clear if these effects are gestationally dependent and novel H_2S donors suitable for clinical drug development remain poorly investigated. NaHS, the most widely used *invitro* H_2S donor, is unsuitable. Recently a novel H_2S generating compound, GYY4137 (GYY) has been developed that slowly

releases H_2S and thus better reflects physiological conditions. We therefore examined effects on myometrial contractility of GYY and NaHS over gestation. **Methods** Myometrial strips from biopsies obtained with consent from women undergoing term elective caesarean sections and hysterectomy biopsies or non-pregnant, 14, 18 or 22 day (term) gestation rats were used. The effects on contractility in response to GYY (1nM-1mM) and 1mM NaHS were examined. **Results** A dose dependent decrease in amplitude was seen with increasing doses of GYY which was significant at 1mM (pregnant human 40%±16, n=5, and term rat, 38% ±6%, n=6, relative to control, 100%). NaHS significantly decreased contraction amplitude (human, 9%± 2%, n=7 and rat, 41% ± 15%, n=6 respectively). The inhibitory effects of GYY and NaHS (1mM) on contractility were gestational dependent, with decreases greatest at term. The effect of GYY and NaHS was significantly greater in pregnant compared to non-pregnant tissue. **Conclusions** NaHS and GYY produce uterine relaxation. GYY and NaHS produce a gestational dependent decrease in contraction with effects most prominent at term, suggesting H_2S could contribute to uterine quiescence until labor onset. This data suggests that H_2S is an attractive target for therapeutic manipulation of human myometrial contractility and that drugs such as GYY may be suitable for this. Ongoing investigations are focussing on elucidating the mechanism by which H_2S donors act.

T-265

Mcl-1 Confers Resistance to Caspase-3 Mediated Apoptotic Cell Death in Human Myometrium. Alyssa Stephenson-Famy,[1] Jason Marks,[2] Arvind Suresh,[2,3] Jeyasuria Pancharatnam,[2,3] Steve N Caritis,[2] Hyagiv N Simhan,[2] Jennifer C Condon.[2,3] *[1]Department of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, Pittsburgh, PA, USA; [3]Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA, USA.*

INTRODUCTION: This study defines the mechanism by which the pregnant human uterus avoids apoptotic cell death despite elevated levels of active caspase-3 (CASP3). Uterine CASP3 has been proposed to act in a tocolytic manner maintaining uterine quiescence during pregnancy. Therefore defining the mechanism by which uterine CASP3 is rendered non-lethal during pregnancy maybe critical in unraveling the factors that define gestational length. We have previously identified in the pregnant mouse uterus that elevated levels of active CASP3 are accompanied by increased anti-apoptotic signaling late in gestation.

HYPOTHESIS: The human pregnant uterine myocyte hosts an anti-apoptotic response which renders CASP3 non-apoptotic permitting the myocyte to harness the tocolytic potential of CASP3 while avoiding apoptotic cell death.

METHODS: We activated CASP3 in primary human pregnant fundal myometrial cultures and the hTERT cell line by ultraviolet irradiation (UV) and Fas ligand (FasL) stimulating both the intrinsic and extrinsic apoptotic pathways. We then suppressed anti-apoptotic signaling utilizing siRNAs to determine the ability of the uterine myocyte to resist apoptotic cell death in the absence of anti-apoptotic signaling.

RESULTS: Stimulation of both intrinsic and extrinsic apoptotic pathways resulted in elevated levels of uterine myocyte CASP3. However apoptotic cell death was restricted to CASP3 activated by intrinsic stimulation via UV. In contrast FasL mediated CASP3 activation was accompanied by increased anti-apoptotic signaling mimicking our in vivo observations in the pregnant mouse uterus. Accordingly suppression of Mcl-1 mediated anti-apoptotic signaling was sufficient to sensitize the uterine myocyte to undergo apoptotic cell death.

CONCLUSION: We have determined that the pregnant human uterine myocyte elevates anti-apoptotic signaling in response to FasL mediated CASP3 activation. These events are sufficient to confer apoptotic resistance on the human uterine myocyte permitting CASP3 to maintain uterine quiescence without resulting in apoptotic cell death.

T-266

Progesterone Regulates Uterine Endoplasmic Reticulum Stress Mediated Caspase-3 Activation and the Timing of Parturition. Arvind Suresh,[1] Jeyasuria Pancharatnam,[1,2] Jennifer Condon.[1,2] *[1]Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA.*

Progesterone (P4) through its receptor (PR) maintains the uterus in a relatively quiescent state throughout pregnancy . We recently proposed that P4 regulated activation of uterine caspase-3 (CASP3) in the pregnant mouse may maintain quiescence by disrupting the myometrial contractile architecture in a reversible and non-apoptotic manner. Towards term, CASP3 levels decline thereby restoring the uterine contractile phenotype and the onset of labor.

This current study demonstrates that uterine CASP3 is likely regulated by an endoplasmic reticulum stress response (ERSR) mounted as a reaction to physiological stress that occurs as the pregnant uterus transitions through gestation. We show that the ERSR signaling cascade is highly modulated by P4 and PR action.

Utilizing pregnant mouse uteri isolated from E11-19, cytoplasmic and nuclear proteins and mRNA were extracted. We detected a balance between activation of ERSR and its resolution as term approaches, which resulted in uterine caspase-3 activation and its decline towards term. On E11-13 elevated levels of ERSR was robustly detected as evidenced by increased phospho-PERK, CHOP and caspase-12 The resolution of ERSR was also identified by increased levels of the chaperone protein BiP that was elevated on E13-16.

Using a mouse model of delayed labor by P4, we discovered that P4 upregulated the ERSR, signified by increased levels of CHOP and decreased BiP thereby leading to an elevation of uterine caspase-3 and a delay in the onset of labor. Uterine tissue taken in 2hr intervals after the injection of the PR antagonist RU486 led to a rapid resolution of the ERSR through an upregulation of BiP and a corresponding downregulation of caspase-3.

These data suggest that the pregnant uterus in response to physiological cues such as hypoxia and mechanical stretch at mid-gestation activates an ERSR and utilizes the resulting caspase-3 activation to maintain uterine quiescence. However as term approaches, the ERSR is resolved through the pro-adaptive anti-apoptotic response and uterine contractility is restored allowing the onset of parturition. The activation and resolution of the ERSR is strongly modulated by P4 and PR action.

T-267

The CRTH2 Agonist Pyl A Inhibits Human Myometrial Contractility in a Mechanism Independent of CRTH2. Lynne Sykes,[1] Hayley Loy,[1] TG Teoh,[2] Phillip R Bennett.[1] *[1]Parturition Research Group, Imperial College, London, United Kingdom; [2]Obstetrics and Gynaecology, Imperial College Healthcare NHS Trust, London, United Kingdom.*

15-deoxy-delta -Prostaglandin J2 delays inflammation induced preterm labour in the mouse and improves pup mortality. The mechanism for this is likely to be via inhibition of NF -κB, inhibiting both pro inflammatory cytokines and labour associated gene expression. 15dPGJ2 is also a CRTH2 (DP2) receptor agonist. We have previously shown that acute administration of 15dPGJ2 has no effect on murine myometrial contractility but that the CRTH2 agonist Pyl A inhibits circular contractility in a mechanism independent of CRTH2. Indomethacin is also a CRTH2 agonist and has been shown to inhibit human myometrial contractility via calcium channel blockade. The effects of acute administration of Pyl A and 15dPGJ2 on human contractility were determined. The *ex vivo* effects of the CRTH2 agonists Pyl A, Indomethacin, 15dPGJ2 and PGD2 on human myometrial contractility was measured by the DMT 800MS myograph. Myometrium from women undergoing elective pre labour caesarean section was dissected and strips were mounted in Krebs (Glucose 2.0g/L, Mg SO_4 0.141g/L, KH_2PO_4 0.16g/L, KCl 0.35 g/L, $NaCl_2$ 6.9g/L, $CaCl_2$ 0.373g/L, $NaHO_3$ 2.1g/L, pH 7.4), gassed with 95% O_2 5% CO_2. Acute cumulative dosing of the agonists was performed (1um-100um) along with vehicle controls. Parameters analysed include total work done by tissue, peak tension, rate of contractility and duration of contractions. Data was analysed using Powerlab software, Excel and SPSS version 18.

Indomethacin reduces contractility with a reduction of total work done from 100% to 47% at 50uM ($p=0.009$). Pyl A reduces contractility with a reduction in total work done from 100% to 31% at 50uM ($p<0.001$). CRTH2 is not expressed on human myocytes, and therefore PGD2 (dual DP1 and DP2 agonist) was used to determine whether the effect of Pyl A is via non specific interaction with the DP1 receptor. No reduction in contractility was seen with PGD2 and 15dPGJ2. The CRTH2 agonist Pyl A inhibits human uterine contractility in a mechanism independent of DP1 and DP2. Further investigation of its mechanism of action may reveal a novel receptor for the inhibition of myometrial contractility.

T-268

The Myometrial Nitroproteome in Pregnancy. Craig Ulrich,[1] Iain LO Buxton.[1,2] *[1]Pharmacology, University of Nevada School of Medicine, Reno, NV, USA; [2]Obstetrics and Gynecology, University of Nevada School of Medicine, Reno, NV, USA.*

The molecular mechanisms involved in uterine quiescence during gestation and those responsible for induction of labor are not completely known. Nitric oxide relaxes uterine smooth muscle in a manner disparate from other smooth muscles

since global elevation of cGMP following activation of soluble guanylyl cyclase does not relax the muscle. S-nitrosylation, the covalent addition of an NO-group to a cysteine thiol is a likely mechanism to explain the ability of NO to relax myometrium. This work is the first to describe the myometrial S-nitrosylproteome in both the pregnant and non-pregnant states. Using the guinea pig model, we show that specific sets of proteins involved in contraction and relaxation are S-nitrosylated in laboring and non-laboring muscle and that many of these proteins are uniquely S-nitrosylated in only one state of the tissue. In particular, we show that S-nitrosylation of the intermediate filament protein desmin is significantly increased (5.7 fold, $p < 0.005$) in pregnancy and that this increase cannot be attributed solely to the increase in protein expression (1.8 fold, $p < 0.005$) that accompanies pregnancy. Elucidation of the myometrial S-nitrosylproteome provides a list of mechanistically important proteins that can form the basis of hypotheses formed to explain the regulation of uterine contraction-relaxation.

T-269

Smooth Muscle Pharmacology of Pregnant vs Non-Pregnant Cervix and Uterus; Search for a Discriminating Agonist. Emma Darios, Stephanie W Watts. *Pharmacology and Toxicology, Michigan State University, East Lansing, MI, USA.*

Because uterine and cervical smooth muscle theoretically function in opposite ways during pregnancy (uterus compliant, cervix constricted), we hypothesized that an agonist existed that would discriminate between uterine vs cervical, and/or pregnant vs non-pregnant smooth muscle function. Isometric contraction of strips of uterine horns and rings of cervix from female virgin and 11±2 day pregnant Sprague-Dawley Rats were measured in an isolated tissue bath and area under oscillations integrated. Agonists were added cumulatively from baseline (contractant) or to half-maximally carbamylcholine-contracted tissues (relaxants). A majority of contractants tested (serotonin receptor agonist 5-hydroxytryptamine, muscarinic cholinergic agonist carbamylcholine, prostaglandin receptor agonist PGF2alpha) stimulated concentration-dependent contraction [rank between uterus vs cervix, pregnant vs non-pregnant (-log EC50 [M] PGF2 alpha =5-HT>carbamylcholine>> KCl)]. By contrast, contraction to oxytocin (a vasopressin/oxytocin receptor agonist) remained in the uterus during pregnancy but was lost in cervices (maximal integrated area as % of KCl contraction; uterus pregnant, uterus non pregnant; cervix pregnant, cervix non-pregnant: 301.5±10.7, 229.0±15.6; 0, 40.2±5.14, $p<0.05$). We next tested relaxants that directly or indirectly stimulated adenylate or guanylate cyclase, dominant relaxant mechanisms in smooth muscle. The adenylate cyclase activator forskolin, beta adrenergic receptor agonists isoproterenol and norepinephrine, and calcitonin receptor agonist calcitonin gene related peptide all caused concentration-dependent relaxation in the uterus vs cervix, pregnant vs non pregnant. In the pregnant cervix, the potency of isoproterenol was reduced ~ 8 fold, that of norepinephrine and CGRP reduced ~2 fold and that of forskolin unchanged. The nitric oxide donor and guanylate cyclase activator sodium nitroprusside relaxed all uterine tissues but relaxation was not observed in any cervices. The differences in pregnant vs non-pregnant (cervical loss of response to isoproterenol) and between uterus and cervix (cervical loss of oxytocin contraction; sensitivity to sodium nitroprusside) validates the utility of this assay. Studies to identify other substances that further discriminate uterine vs cervical function and changes in pregnancy are crucial for understanding mechanisms that could halt preterm labor.

T-270

Splice Variants of the K2P Channel TREK-1 Associated with Preterm Labor. Yi-Ying Wu,[1] Cherie A Singer,[1] Iain LO Buxton.[1,2] *[1]Pharmacology, University of Nevada School of Medicine, Reno, NV, USA; [2]Obstetrics and Gynecology, University of Nevada School of Medicine, Reno, NV, USA.*

Spontaneous preterm labor (PTL) is a uniquely human problem that results in preterm delivery of an underdeveloped fetus, and the causes are unknown. TREK-1, a member of the two pore domain potassium channel family, plays an essential role in setting the resting membrane potential. We have shown that TREK-1 is up-regulated during pregnancy toward term for maintaining uterine quiescence by hyperpolarizing the cell membrane. Our hypothesis is that TREK-1 plays an essential role in human myometrial relaxation during pregnancy and splice variants of the TREK-1 channel are associated with PTL. Using RT-PCR, we have identified five unique TREK-1 splice variants in preterm pregnancy tissue. The unique myometrial TREK-1 variants lack either the pore or transmembrane domains, or both. Flag tag wild type TREK-1 was cloned into the MCS site of the pCDH-CMV-MCS-EF-1-GFP vector and stably expressed in HEK293T cells to characterize function and localization. 6xHis tag splice variants were cloned into the MCS site of the pCDH-CMV-MCS-EF-1-RFP vector and expressed in HEK293T cells. The unique 6xHis tag spice variants will be expressed in HEK293T either lacking or containing native TREK-1 in order to characterize variant channel function electrophysiologically and interaction with wild type TREK-1. Based on nucleotide sequences, splice variant 1 may translate into a potassium channel with a single pore domain and single trans-membrane domain. Splice variant 2 and 4 may translate into proteins with single transmembrane domains. Both splice variant 2 and 3 may translate into an 11 amino acids peptide with partial N-terminal domain. Splice variant 5 may translate into a 21 amino acid peptide with partial N-terminal domain. We have isolated a unique myometrial TREK-1 variant from preterm myometrium by anti-TREK-1 antibody. Also, we have found that splice variant 2 and 4 can be translated into two distinct 18kDa proteins which still have a wild type C terminus which can be detected by anti-TREK-1 antibody. Supported by March of Dimes Prematurity Initiative Grant 21-FY10-176, NIH HD053028, and a grant from the Bill and Melinda Gates foundation to ILOB.

T-271

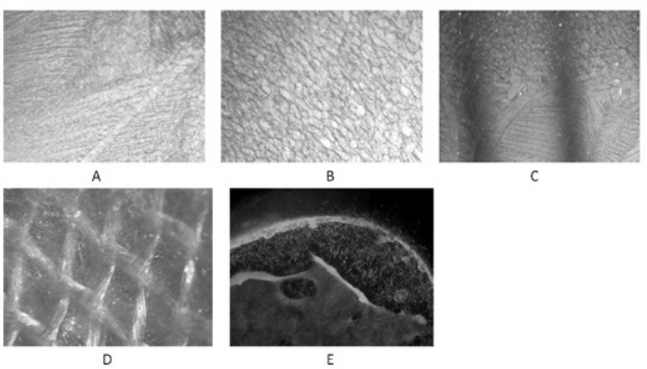
Creating a Physically Robust Neo-Tissue with Synthetic and Natural Scaffolds. Roger C Young, Gabriela Goloman. *Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA.*

Objective: To create a physically robust neo-tissue composed of human uterine myocytes living on a mixed synthetic/natural scaffold.

Methods: *Scaffolds.* Squid pen chitosan (60% deacetylated) was purchased from Industrial Research Ltd., New Zealand, and dissolved in acetic acid. Collagen from 26 rat tails was extracted, sterilized and dissolved in acetic acid, then neutralized with sodium phosphate. Mixtures of chitosan and collagen were prepared in different volumetric ratios. As detailed below, the primary scaffolds were created using different freezing techniques. The primary scaffolds were freeze-drying at -105^0 C, exposing the specific matrix. In some experiments vicryl mesh was coated with chitosan solution, frozen, and freeze-dried.

Cells. Human uterine myometrium was obtained from pregnant women at the time of Cesarean section, primary cell lines were begun and maintained in culture. Third passage myocytes were cultured onto the scaffolds to create the neo-tissues. Live/dead assays were performed using the cytotoxicity assay from Invitrogen (Eugene, Oregon).

Results:



A. Lateral freezing using metal plate with edge in liquid N_2. Note channeling.
B. Freezing performed from bottom-up. Note honey-comb pattern.
C. Combined lateral and bottom-up techniques showing interface of channels and honeycomb. Dark lines are 1 mm markers.
D. Vicryl mesh with chitosan creating a physically strong synthetic matrix.
E. Live-dead assay of cells cultured for 12 days on chitosan-collaged (2:3). Note retraction of the outer sheet of cells.

Conclusion: Using different chitosan-collagen mixtures and freezing techniques, it is possible to structurally customize matrices that are capable of supporting growth of human uterine myocytes. It is also possible to combine vicryl mesh with these mixtures to create a mobile, physically robust structure that should be capable of temporary structural support post-transplantation.

T-272

Inward Rectifier Potassium Channels Pace Contractions in Pregnant Rat Myometrium. Roger C Young, Gabriela Goloman. *Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA.*

Objective: Electrical activity is important in triggering each contraction, but only one method is known to change contraction frequency by targeting ion channel function - blocking the inward rectifier potassium channel (Kir) with 50 μM Ba^{2+} increases contraction frequency. Previously we have reported

Kir currents in isolated rat and human uterine myocytes. This work seeks to establish the tissue-level mechanisms of action of Kir.

Methods: Rat myometrium was obtained from d 20 pregnant rats. Isometric contractility experiments were performed. Contact electrodes were used to record bioelectrical signals, allowing precise measurement of the onset and offset of each contraction. 50 μM Ba^{2+} was used to block Kir channels; 300 μM 4-AP to block Kv channels; and 100 nM apamin to block SK channels. Experiments were performed in baths containing 2, 4.8 or 15 mM KCl. In other experiments, wortmannin was used to reduce tissue motion, and intracellular microelectrodes were used to measure membrane potentials and action potentials before and after Kir block.

Results: Ba^{2+} exposure increased contraction frequency of rat myometrium in baths containing 2 mM (96% increase; n=5, p=.02) and 4.8 mM (60%; n=5, p=.05), but not 15 mM KCl. The durations of the contractions were unchanged by Ba^{2+} at any bath K^+. Ba^{2+} increased contraction frequencies even in the presence of 4-AP and apamin, indicating the effects of Ba^{2+} are not due to Kv or SK block. Microelectrode studies (n=3)performed in the presence of wortmannin showed Ba^{2+} shortened the time between action potential bursts, confirming the contractility data, but also indicating that Kir does not function primarily through a mechanotransduction mechanism. Ba^{2+} did not change the spiking action potential, and, surprisingly, also did not depolarize the resting potential.

Conclusions: 50 μM Ba^{2+} specifically targets Kir channels, which function to prolong the time between contractions. There are two possible explanations for the failure of Ba^{2+} to depolarize the resting potential. 1) Kir is one of many hyperpolarizing currents that contribute to the tissue-level resting potential. Hence, Kir block does not depolarize the tissue, but makes depolarization more favorable. 2) Inhomogeneous Kir distribution results in regional pacemakers that are primarily affected by Kir block, but are not recorded by microelectrode measurements. Further work will be required to confirm these mechanisms.

T-273

Nuclear Factor-Kappa B Is Required for Oxysterol-Mediated Inflammatory Activation in Placental Trophoblasts. Irving LMH Aye,[1] Brendan J Waddell,[2] Peter J Mark,[2] Jeffrey A Keelan.[1] *[1]School of Women's and Infants' Health, University of Western Australia, Australia; [2]School of Anatomy and Human Biology, University of Western Australia, Australia.*

Objectives: We previously demonstrated that oxysterols promote pro-inflammatory cytokine secretion in placental trophoblasts by a TLR4-dependent mechanism. In this study, we investigate the involvement of nuclear factor-kappa B (NF-κB) in oxysterol-induced inflammation in cultured trophoblasts.

Methods: Primary human trophoblasts were isolated from term placentas by dispase enzyme digestion, purified over Percoll and allowed to syncytialize for 4 days in vitro. Cells were then exposed to oxysterols (25-hydroxycholesterol [25-OHC] or 7-ketocholesterol [7-ketoC]) or lipopolysaccharide (positive control) in the presence/absence of selective inhibitors of IKK activity (parthenolide and TPCA-1). Inflammatory activity was determined by measuring the concentration of proinflammatory cytokines (IL-6, MIP-1β and TNF-α) in culture media. NF-κB activity was evaluated by examining phosphorylation of NF-κB subunit p65/RelA (at Ser536), as well its nuclear translocation.

Results: Treatment with 25-OHC and 7-ketoC increased p65/RelA (Ser536) phosphorylation by 2-3 fold ($P<0.05$), and led to increased p65/RelA nuclear translocation by up to 4 fold ($P<0.001$). Inhibition of the IKK complex formation with parthenolide attenuated both 25-OHC- and 7-ketoC- induced p65/RelA (Ser536) phosphorylation ($P<0.05$); while IKK-β inhibition using TPCA-1 did not affect p65/RelA (Ser536) phosphorylation. Both inhibitors however, decreased the oxysterol-mediated production of all cytokines examined ($P<0.05$).

Conclusions: These findings indicate that oxysterols promote placental inflammation via NF-κB dependent mechanism.

T-274

Can Cytokines Predict Outcome in Threatened Miscarriage? Jean Calleja-Agius,[1,3] Shanthi Muttukrishna,[2] Eric Jauniaux.[1] *[1]Obstetrics and Gynaecology, Institute for Women's Health, University College London, London, United Kingdom; [2]Obstetrics and Gynaecology, Anu Research Centre, University College Cork, Cork, Ireland; [3]Anatomy, University of Malta, B'Kara, Malta.*

Objective: To evaluate circulating and intracellular levels of Th-1 and Th-2 cytokines in women presenting with threatened miscarriage (TM) and subsequent outcome.

Study design: Plasma levels of TNF-receptors 1 and 2, TNFα, IFNγ, and interleukins IL-6 and IL-10 were measured using flowcytometric bead assays in 80 women with TM, including 53 with normal outcome and 27 who miscarried. Fluorescent antibody-labeling was also performed on whole blood in a subgroup of 27 TM cases, including 16 with normal outcome and 11 who miscarried.

Results: Monocyte expression of TNFα and circulating levels of TNFα, IFNγ, IL-10, IL-6 and TNF-R1 were significantly lower whereas circulating levels of TNFα/IL-10, IFNγ/IL-10 and TNFα/IL-6 ratios were significantly higher in TM cases who subsequently miscarried compared to those with normal outcome.

Table 1 - Comparison of circulating cytokine and receptor levels in the plasma in TM with normal pregnancy outcome and TM who subsequently miscarried. T-test showed that there were statistically significant elevated levels of TNFα, IFNγ, IL-10, IL-6 and TNF-R1 in TM who proceeded to livebirth, while TM who miscarried had statistically higher ratios of TNFα/IL-10, TNFα/IL-6 and IFNγ/IL-10

TNFα	<0.001 *
IFNγ	<0.05 *
IL-10	<0.001 *
IL-6	<0.005 *
TNF-R1	<0.05 *
TNF-R2	NS
TNFα/IL-10	<0.001 *
TNFα/IL-6	<0.05 *
IFNγ/IL-10	<0.001 *

The best combination of biomarkers for miscarriage was TNF-R2 and TNFα, which gave a positive predictive value of 66%, sensitivity of 54% and specificity of 92%.

Conclusion: An increased Th-1 type of immune response was found in TM cases complicated by a subsequent miscarriage. This may prove to be a useful clinical investigation when couselling patients presenting with TM.

T-275

Proteomics of Preeclamptic Placenta. Monique V Chireau,[1] J Will Thompson,[2] Brenna Richardson,[2] Arthur Moseley,[2] L Kristin Newby.[3] *[1]Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA; [2]Institute for Genome Sciences Policy, Duke University Medical Center, Durham, NC, USA; [3]Cardiology, Duke University Medical Center, Durham, NC, USA.*

Introduction: Preeclampsia (PE/E) is a major cause of maternal and fetal mortality and morbidity. Research implicates proteins produced by the placenta in the pathogenesis of PE/E, including VEG-F, s-flt, endoglin and PGF. Prior studies have identified as many as 296 proteins from 293 genes in placenta. Proteomic analysis may identify proteins that are differentially expressed in women with and without PE/E, and shed light on PE/E's underlying pathophysiology.

Methods: Placentas were collected from 5 preeclamptic and 4 normal women. A dual TRIzol (guanidium-thiocyanate-phenol-chloroform) protocol was used to generate TRIzol-soluble and sonication-soluble fractions from each patient, which were analyzed using two-dimensional liquid chromatography-mass spectrometry/mass spectrometry (LC/LC-MS/MS).

Results: 2051 proteins from 1977 unique genes were identified from placental samples, 1360 from the TRIzol fraction and 1417 from the sonication fraction. The TRIzol-soluble fraction was found to contain soluble and organellar proteins. The sonicated fraction contained a higher proportion of cytoskeletal and membrane proteins. After correction for multiple testing using Hochberg FDR, 46 proteins in the TRIzol fraction and 19 proteins in the sonicated fraction were differentially expressed between preeclamptic and non-preeclamptic women (alpha < 0.05). VEG-F receptor 1 and endoglin were identified in both TRIzol and sonication fractions, however, VEG-F and PGF were not. Functional analysis using Ingenuity Pathway Analysis documented that differentially-expressed proteins are found in pathways related to extracellular matrix remodeling, immune function, invasion and cell signaling.

Conclusions: Comprehensive quantitative proteomic analysis of placenta identifies proteins not previously noted in this tissue, some of which are expressed differentially in PE/E. VEG-F and PGF were not identified. Differentially expressed proteins appear to be part of matrix remodeling, invasion and cell signaling pathways, similar to those noted in cancer. Although it appears that these proteins may discriminate between preeclamptic and non-preeclamptic women, further research is needed to explore these associations and to investigate PE/E's underlying pathophysiology.

T-276

Differential Expression of Glucocorticoid Receptor Isoforms in Human Placenta. Zarqa Saif, Annette Osei-Kumah, Vicki L Clifton. *Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adealaide, SA, Australia.*

We have identified that in the presence of maternal asthma, human female placentae are more sensitive to glucocorticoids than male placentae which does not appear to be mediated by glucocorticoid receptor alpha (GRα) isoform.

This evidence had raised the question of whether sensitivity to glucocorticoids may be conferred by the presence of other GR isoforms. Different isoforms of GR have been previously described (GRα, GRβ, GRγ and GR-P) originating from splice variants of the GR gene. However there are also different isoforms of GRα including GRα-A, GRα-B, GRα-C1-C3 and GRα-D1-D3 that originated from alternate translation initiation sites. We hypothesise that sex specific differences in glucocorticoid sensitivity are related to the different GR isoforms in the placenta and by the cellular location of GR in either the cytoplasm or nucleus. This study aimed to identify the differential expression of GR isoforms in the human placenta in relation to the presence of maternal asthma and fetal sex. Placentae were collected from pregnancies complicated by asthma (n=10) and compared to healthy control placentae (n=10). Cytosolic and nuclear protein fractions were prepared and GR isoforms detected using western blot and relative expression measured by densitometry. We have identified the presence of 84kDa and 81kDa bands corresponding to GRα and GRβ, isoforms. We also detected other bands corresponding to 75kDa, 65kDa and 50kDa. These bands were specific for GRα-C, GR-P and GRα-D respectively. GRα and GRβ expression increased in female placentae in the presence of maternal asthma but only GRβ increased in the nucleus of male placentae from pregnancies complicated by asthma. GR-P and GRα-C were increased in male placentae relative to female placentae of asthma pregnancies. These data suggest there are multiple isoforms of the GR in the placenta that vary with fetal sex and maternal asthma. Insensitivity to glucocorticoids in the male placenta may be conferred by increased expression of GRα-C and GR-P in the nucleus.

T-277

Glucocorticoids (GCs) Specifically Up-Regulate CD163 Expression in Fetal Hofbauer Cells (HBCs). Seth Guller,[1] Zhonghua Tang,[1] Tracy Niven-Fairchild,[1] Serkalem Tadesse,[2] Errol R Norwitz.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine; [2]Obstetrics and Gynecology, Tufts Medical Center.*

Background: Although GCs are administered routinely to women at risk for preterm birth at < 34 weeks of gestation, little is known about their effect on the placenta. Placental explants and primary cultures of HBCs (fetal macrophages) were used to test the effects of GC on levels of CD163, a macrophage-specific heme-scavenging protein, *in vitro*.

Methods: HBCs were isolated from term placentas using Percoll gradients and negative immunoselection (n=4). Levels of CD163 and another macrophage-specific marker, folate receptor-β (FR-β), mRNA and protein were determined by quantitative real-time PCR (qRTPCR) and Western blotting (WB) respectively, with and without treatment with GC using either cortisol (Cort) or dexamethasone (DEX) at concentrations of 1-100 nM for 4-48 h. Effects of GC treatment on expression of CD163 in placental explant cultures (n=4) was examined by qRTPCR and by *in situ* hybridization (ISH) using digoxigenin-labeled sense and anti-sense probes generated by PCR.

Results: Treatment of HBCs with Cort or DEX for 24 h enhanced CD163 protein levels 11- and 13-fold, respectively (P<0.001) by WB, although DEX was 10-fold more potent in this regard than Cort with dose-dependent effects noted bewteen 1 and 10 nM and between 4 and 48 h of treatment. Conversely, GC treatment did not significantly affect FR-β protein expression. Treatment of HBCs with 100 nM estradiol, progesterone, and testosterone had no effect on CD163 or FR-β levels. qRTCR revealed a 24 h treatment with 100 nM cortisol or DEX up-regulated CD163 mRNA expression in HBCs 10- and 15-fold, respectively (P<0.001), whereas effects on FR-β mRNA were ≤ 2-fold. Similarly, DEX treatment of explant cultures for 4 to 24 h stimulated CD163 mRNA levels up to 10-fold (P<0.001). No significant effects on FR-β expression were noted. A DEX-dependent increase in CD163 mRNA levels in HBCs was noted in explant cultures using ISH.

Conclusions: CD163 levels in HBCs are exquisitely sensitive to and specifically up-regulated by GC treatment. Based on the proximity of HBCs to the fetal vasculature, modulation of CD163 expression in this cell type could be used to assess fetal/placental exposure to bioactive steroid.

T-278

Circulating IGF-I and Placental Type 1 IGF Receptor in Normal and Gestational Diabetic Pregnancies. Nicholas P Illsley,[1] Chistopher CK Leung,[1] Stacy Zaumdio.[2] *[1]Obstetrics, Gynecology and Women's Health, UMDNJ-New Jersey Medical School, Newark, NJ, USA; [2]Obstetrics and Gynecology, Hackensack University Medical Center, Hackensack, NJ, USA.*

Introduction: Despite a number of studies, the role of the insulin-like growth factor (IGF) system in regulating human fetoplacental growth is still far from clear. In this report we investigated the role of the IGF system in normal and gestational diabetic pregnancies. We hypothesized that fetal circulating IGF-I and type 1 IGF receptor (IGFIR) expression on the placental syncytial membranes would be higher in diabetic pregnancies. **Methods:** Cord blood and placental tissue were obtained from term normal and insulin-treated gestational diabetic (GD) pregnancies. IGF-I was measured by ELISA while purified syncytial microvillous and basal membranes were used to measure IGFIR by Western blotting. **Results:** Fetal IGF-I concentrations were not different in GD compared to normal pregnancies (185±12 vs 161±12 ng/mL; n=20, p<0.001; unpaired t test). Birth weight and placental weight were positively correlated with circulating IGF-I in the normal pregnancies (r^2=0.38, 0.54; p<0.01, n=20) however this relationship was lost in the GD pregnancies. Expression of microvillous and basal membrane IGFIR was increased by 60±23 and 51±4% in GD compared to normal pregnancies (p<0.001, n=34, 27). Basal membrane (but not microvillous) expression of IGFIR demonstrated a negative correlation with both birth and placental weight (r^2=-0.18, -0.20, p<0.05, n=23) in GD, paralleling the relationship between basal membrane IGFIR and circulating IGF-I (r^2=-0.54, p<0.001). **Conclusions:** Although mean IGF-I concentrations were not different, the positive correlations between fetal circulating IGF-I and birth and placental weights suggest an important regulatory role. The loss of this relationship in gestational diabetics may be related to the reduced expression of basal membrane IGFIR at higher levels of circulating IGF-I, and points to the significant role of the the type 1 IGF receptor as well as IGF-I itself.

T-279

Modulation of the Endocannabinoid System (ECS) in Plasma and Trophoblast after RU486 Administration. Tulay Karasu, Timothy H Marczylo, Patricia M Lam, Emeka Oloto, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences, CSMM, University of Leicester, Leicester, United Kingdom.*

Introduction: Endocannabinoids (EC) such as Anandamide (AEA) are ligands for EC receptors (CB1, CB2). AEA is detrimental for implantation and early pregnancy and ECS dysregulation adversely effects pregnancy outcome. Progesterone (P4), essential for pregnancy maintenance, interferes with the ECS. We investigated the effect of Mifepristone (RU486), a P4 antagonist used to initiate medical termination of pregnancy (MTOP), on the ECS in plasma and trophoblast of women undergoing MTOP.

Methods: AEA, and two congeners (OEA, PEA) were measured by UHPLC-MS/MS. Blood was taken pre- and 1 day after RU486 administration to 66 women with healthy first trimester pregnancies. Trophoblast was collected from MTOP women and women undergoing surgical TOP (STOP) as controls. mRNA was measured by qRT-PCR.

Results: Plasma AEA, OEA and PEA were significantly increased after RU486 administration.

Plasma EC levels (nM, mean±SD) of MTOP patients

	Pre-RU486	Post-RU486	p-value
AEA	0.53±0.03	0.60±0.03	0.003
OEA	2.97±0.15	3.30±0.14	0.049
PEA	6.97±0.50	8.96±0.67	0.006

Trophoblast AEA, OEA and PEA were significantly increased in MTOP compared to STOP.

Trophoblast levels (pmol/g, mean±SD) of MTOP patients

	MTOP n=9	STOP n=14	p-value
AEA	7.99±1.39	4.38±0.62	0.018
OEA	35.4±4.3	20.7±3.0	0.009
PEA	101.2±7.2	57.9±6.4	0.012

The trophoblast transcript levels of the AEA metabolizing enzymes NAPE-PLD and FAAH in MTOP were significantly increased compared to STOP (p=0.0002 and p=0.029). CB1 and CB2 transcripts were increased in MTOP trophoblast, but did not significantly (p=0.1 and p=0.08).

Conclusion: Plasma and trophoblast AEA, OEA and PEA are significantly increased after RU486 administration possibly via modulation of enzyme levels.

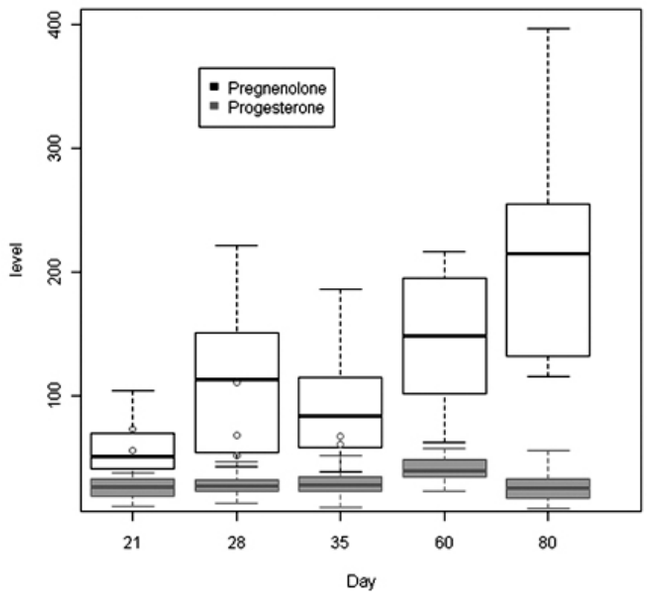
T-280

Pregnenolone and the Oocyte Donation Model: New Insights into the Luteoplacental Shift. Frederick L Licciardi, Cheongeun Oh. *OBGYN, NYU School of Medicine, NYU Langone Medical Center, New York, NY, USA.*

The timing of the luteoplacental shift has been eloquently studied,however there has been little new information brought forth on the subject over the past 2 decades.

OD provides a unique model to study this phenomenon,as women who become pregnant do so in the absence of a CL.The timing of the LPS in oocyte recipients using changes in progesterone(Prog) levels can only be extrapolated because exogenous Prog is always given. Pregnenolone(Preg) is the Prog precursor

made by the placenta and is without an exogenous source. Measurements of Preg may provide better information about the timing and even the quality of placental maturation.
Methods.Recipients' serum was drawn on luteal days 21,28,35,60and80.Prog was measured using the Immulite system,an immunoassay utilizing polystyrene bead solid phase and a chemiluminescent substrate.The intra and inter assay imprecision is 7-10% and 9-12%. 50 delivered patients were selected, 36 of whom had Prog levels for all days listed.
Stored frozen serum samples from 6 delivered DE recipients were used for the Preg analysis.This number was low due to the high cost of each test.Analysis was performed by Endocrine Sciences using liquid chromatography separation with tandem mass spectrometric detection (LC-MS/MS). An MDS-Sciex API5000 triple quadrupole mass spectrometer was used and quantification of analyte and standard was performed in selected reaction monitoring mode(SRM).The intra-day imprecision is 2-3%.Using random effects models to fit to the longitudinal data, the significant differences between Preg and Prog were evaluated.
Results. Preg levels rise in the very early DE pregnancy, and this rise is significantly different from the changes in Prog($p<.05$ linear mixed model).



Box plots at individual days respect to their longitudinal measurements.
Concl.These results provide valuable new information and ignite interest in further study of the developing placenta.Future work related to the timing and amplitude of this rise in pregnenolone can allow us to better characterize the luteoplacental shift. Pregnenolone may potentially be a biomarker of fetal health and chromosomal status.

T-281

Circadian Variation in Placental Expression of Inflammatory Mediators.
Peter J Mark, Michaela D Wharfe, Jessica L Lewis, Brendan J Waddell. *School of Anatomy & Human Biology, The University of Western Australia, Perth, Western Australia, Australia.*
Objectives: Rhythmic expression of clock genes drives circadian variation in various physiological processes both centrally and in peripheral tissues such as the liver. We recently demonstrated the rat placenta expresses all canonical clock genes in a highly zone-specific pattern and with some circadian variation. Placental inflammatory status influences the onset of parturition, the timing of which shows a distinct circadian pattern in the rat, and systemic levels of pro-inflammatory cytokines also exhibit circadian variation. Therefore, we tested the hypothesis that placental expression of inflammatory mediators varies in a circadian manner.
Methods: Pregnant rats (n=6/time point) were sampled over days 21-22 of gestation (term = day 23). Samples of junctional (JZ) and labyrinth (LZ) zones of the placenta were collected at 0800, 1400, 2000 and 0200 h, which equate to zeitgeber times ZT1, ZT7, ZT13 and ZT19 respectively. JZ and LZ expression of mRNA encoding pro-inflammatory mediators (TNF-α, IL-6, IL-1β, Cox1 and Cox2) and an anti-inflammatory circadian regulator (Sirt1) were measured by RT-qPCR.
Results: LZ expression of TNF-α, IL-6, Cox2 and Sirt1 all varied with time-of-day. Specifically, peak TNF-α expression at ZT1 was 2.2-fold higher than its trough at ZT13 (P=0.034). IL-6 expression was 2.3-fold higher at ZT19 than at ZT7 (P=0.004), while Cox2 expression at ZT13 was 2-fold higher than at ZT7. Sirt1 expression at ZT13 was 1.6-fold higher than at ZT1 (P=0.05). Circadian variation was less prevalent in the JZ, although TNF-α and IL-1β expression were 1.6-fold (P=0.021) and 2.4-fold (P=0.038) higher, respectively, at ZT19 than at ZT7.
Conclusion: In summary, these data show that the placenta exhibits circadian variation in expression of pro-inflammatory mediators late in gestation, with cytokine expression highest late in the active (dark) phase and early in the inactive (light) phase. This circadian pattern of placental inflammatory status is likely to influence placental physiology and the timing of parturition.

T-282

Effect of VEGF Overexpression by Conception Maternal VEGF in Mice.
Abhijeet Minhas,[1] Shannon Bainbridge,[2] Dawei Qu,[1] Hoon-Ki Sung,[1] Andras Nagy,[1,3] S Lee Adamson.[1,2,4] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital; [2]Interdisciplinary School of Health Sciences, University of Ottawa; [3]Department of Molecular Genetics, University of Toronto; [4]Department of Obstetrics & Gynecology, University of Toronto, Toronto, Canada.*
Objective: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor expressed by all organs, including the highly vascular placenta. It increases in the maternal circulation during pregnancy, but its source and function are unknown. It was hypothesized that if the placenta was the source of increased VEGF in the maternal circulation during pregnancy, then circulating VEGF in wildtype mothers carrying $Vegf^{hi}$ concepti would be elevated relative to controls.
Methods: Male $Vegf^{hi}$ mice were mated with wildtype (WT) CD1 females to obtain pregnancies where approximately half the concepti carried the $Vegf^{hi}$ transgene. Maternal plasma, maternal organs and regionally-enriched placental samples (junctional zone, labyrinth, and chorionic plate) were collected at E17.5 from WT pregnancies and from CD1 female mice (aged 8-12 weeks) mated with $Vegf^{hi}$ males. $VEGF_{120/164}$ protein was measured in the maternal circulation, organs, and the placenta by ELISA.
Results: When WT female mice were mated with $Vegf^{hi}$ males, placentas overexpressed VEGF protein compared to WT pregnancies ($p<0.05$). However, litter size (13.6±1.4 vs. 13.9±2.1 in WT, $p>0.05$; N= 8 and 10 respectively) and maternal body weight (63±2 vs. 61±2 g in WT, $p>0.05$; N=8 and 16 respectively) in the WTx$Vegf^{hi}$ cross were not different compared to WT pregnancies. Even though VEGF was overexpressed in half the concepti, and consequently their placentas, we surprisingly found that maternal systemic arterial circulating levels of VEGF decreased relative to controls (303±62 vs. 599±48 pg/ml in WT, $p<0.05$; N=7 and 14 respectively). WT females mated with $Vegf^{hi}$ males had kidney and decidua VEGF levels that were not different than controls. However, ovarian VEGF levels were significantly decreased (320±68 vs. 536±57 pg/mg of total protein in WT, $p<0.05$; N=10 in both groups).
Conclusion: Contrary to our hypothesis, we did not obtain evidence supporting the placenta as a direct contributor to maternal plasma VEGF. However, conceptus VEGF appears to indirectly influence maternal plasma VEGF possibly by affecting ovarian VEGF production.
Supported by CIHR operating grant MOP-93618.

T-283

Acute Effects of Polyinosinic:Polycytidylic Acid (PolyIC) and Lipopolysaccharide (LPS) on Mouse Placental and Fetal Blood-Brain Barrier (BBB) Multidrug Resistance Phosphoglycoprotein (P-gp) Function.
Sophie Petropoulos,[1] Majid Iqbal,[2] Melanie C Audette,[2] Stephen G Matthews,[2] William Gibb.[3] *[1]Pharmacology and Therapeutics, McGill University; [2]Physiology, University of Toronto; [3]Ob-Gyn, Cellular and Molecular Medicine, University of Ottawa.*
The placenta is the primary barrier between maternal and fetal circulation. However, the placenta is not impermeable and xenobiotics may traverse the placenta, potentially exposing the fetus to teratogenic factors. The fetal brain is particularly susceptible to many xenobiotics and is protected by the BBB. We have previously shown that P-gp in the placenta and fetal BBB protects the developing fetus by limiting transfer of substrates found in maternal circulation. Inhibition of placental and fetal BBB P-gp may inadvertently expose the developing fetus to xenobiotics and endobiotics in maternal circulation. Maternal and intra-amniotic infections occur during pregnancy and account for up to 40% of preterm births. Infection has been previously shown to decrease the

expression of P-gp mRNA in the near term rat placenta; suggesting a regulatory role of cytokines on P-gp. In this study, we examined the effects of PolyIC (simulating viral infection) and LPS (simulating bacterial infection) on P-gp function in the placenta and fetal BBB during mid-gestation. Methods: Pregnant C57BL/6 mice (n=4-6; E15.5) were injected (i.p.) with either 150µg/kg of LPS or 5mg/kg PolyIC or vehicle (saline). [^{3}H]Digoxin (50µg/kg; substrate of P-gp) was injected (i.v.) 3hrs post-treatment and animals were euthanized 1hr later. Maternal plasma, 'fetal-units' (fetal membranes, amniotic fluid and whole fetus), and fetal brains were collected. Maternal pro-inflammatory cytokine, IL-6, was measured by ELISA. Results: PolyIC and LPS significantly elevated circulating maternal IL-6 ($P<0.01$ and $P<0.001$, respectively). However, neither PolyIC or LPS significantly increased accumulation of [^{3}H]digoxin in the 'fetal-unit' or fetal brain, suggesting no effect of acute (4h) infection on P-gp function. Conclusion: Acute viral or bacterial infection occurring during mid-gestation does not significantly impact placental and fetal BBB P-gp mediated protection. This is important, given the high incidence of maternal infection during pregnancy. However, it is possible that longer term treatment with PolyIC and LPS (mimicking chronic infection) may induce changes in P-gp at these two important barrier sites.

T-284

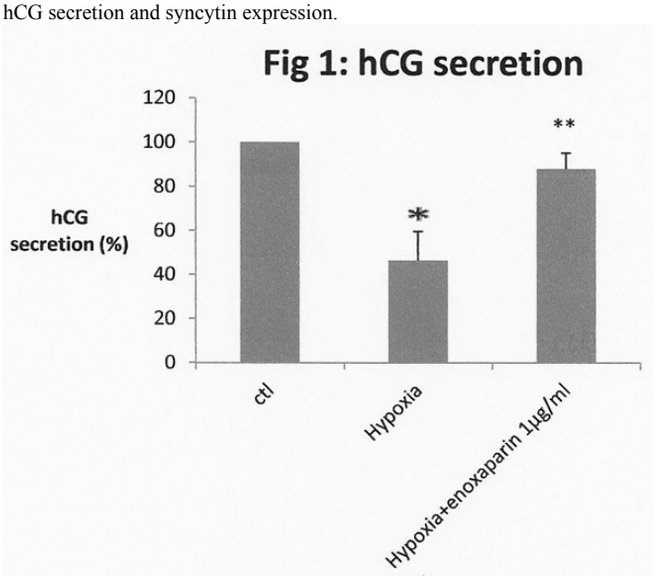
A Favorable Effect of Low Molecular Weight Heparin on Trophoblasts Exposed to Hypoxia. Eli Rimon, Joseph B Lessing, Michael Kupferminc. *Obstetrics and Gynecology, Tel Aviv Medical Center, Tel Aviv University, Tel Aviv, Israel.*

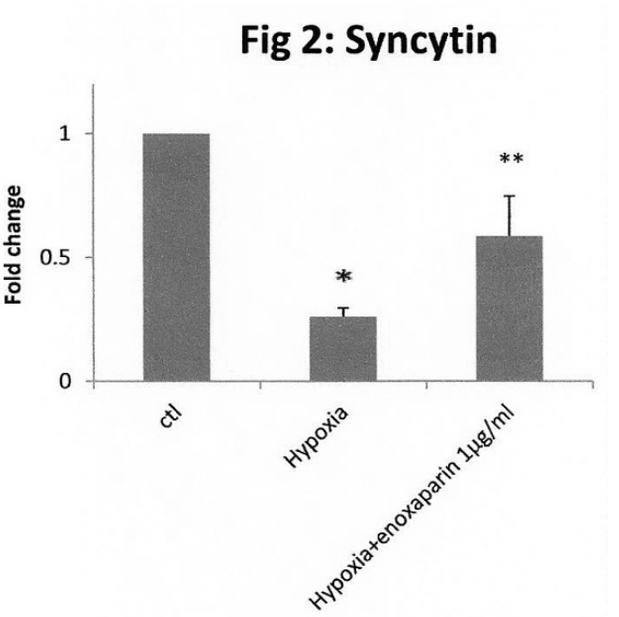
Introduction: **Sever**al studies have shown a favorable effect of Low molecular weight heparin (LMWH) on pregnancy outcome. This effect was attributed mainly to its anticoagulant effect but recent studies have shown a direct effect of LMWH on trophoblasts.

Objective: to investigate the effect of LMWH on trophoblasts exposed to hypoxia.

Methods: Jeg-3 cells were cultured for 24 hours in either standard conditions (O2=20%), hypoxia (O2< 1%) or in hypoxia with Enoxaparin (LMWH 1 µg /ml). In order to investigate the effect of Enoxaprine on trophoblasts differentiation we measured the secretion of human chorionic gonadotropin (hCG) to culture medium and expression of Syncytin. In addition, we investigated the effect of Enoxaparine cleavage of poly (ADP-ribose) polymerase (PARP) and p53 expression as markers of trophoblasts apoptosis.

Results: hCG secretion and expression of Syncytin were reduced significantly when trophoblasts were exposed to hypoxia. Interestingly, Enoxaparin significantly protected JEG3 cells from hypoxic injury as expressed by increased hCG secretion and syncytin expression.





Hypoxia increased the cleavage of PARP and P53 expression. Importantly, addition of Enoxaparin increased the number of cells survived, reduced PARP cleavage and reduced expression of P53 by approximately 2 folds.

Conclusions: Our preliminary data suggest that Enoxaparin protects trophoblasts from hypoxic injury by its favorable effect on trophoblasts differentiation and prevention of apoptosis. The data suggest a possible mechanism by which Enoxaparin prevents placental hypoxic injury and finally prevents pregnancy complications.

* A Comparison of hypoxia and control $P<0.05$

** A Comparison of hypoxia and hypoxia with Enoxaparin, $p<0.05$

T-285

Differential Uterine and Systemic Sensitivity to BK_{Ca} and K_V Channel Inhibition after Estradiol-17β (E2)-Induced Uterine Vasodilation in Nonpregnant (NP) Ewes. CR Rosenfeld, T Roy. *Pediatrics, UT Southwestern Medical Center, Dallas, TX, USA.*

Background. E2 increases NP uterine blood flow (UBF) >4-fold by 90min and remains elevated for >2h in the absence of altered mean arterial pressure (MAP) due to increased heart rate (HR) and cardiac output. The rise in UBF reflects enhanced nitric oxide synthesis and activation of large conductance Ca^{+2}-activated (BK_{Ca}) and voltage-activated (K_V) K^+ channels; it is unclear if they contribute to maintenance of UBF and if they act differently in uterine and systemic vasculature. **Objective.** Compare effects of local uterine BK_{Ca} and K_V inhibition on uterine and systemic hemodynamic variables after E2-induced vasodilation. **Methods.** 5 NP ewes were ovariectomized, flow probes placed on main uterine arteries (UA), and catheters implanted in vena cava, distal aorta and UA to infuse inhibitors and uterine vein to sample blood. Ewes received daily E2 (1ug/kg iv) until maximum UBF achieved. Ewes then studied with E2 alone (C, n=11) or E2 plus local UA BK_{Ca} or K_V inhibition with tetraethylammonium (TEA, n=5; 0.4.-0.8 mM) or 4-aminopyridine (4AP, n=5; 0.01-0.08 mM), respectively. Inhibitors were infused intra-arterial for 30min at maximum UBF, i.e., 90min after E2-infusion. MAP, HR and UBF were continuously monitored. A-V samples from 3 E2 studies, 2 E2+TEA and 2 E2+4AP were assayed for cGMP. Uterine vascular resistance (UVR) was calculated; data are means±SEM. **Results.** Hemodynamic variables before E2 did not differ in groups ($P>0.1$). 90min after E2, C UBF rose (39±4.3 to 175±72 ml/min, $P<0.001$), UVR fell (2.36±0.4 to 0.45±0.03 min·mmHg/ml, $P<0.001$), and HR fell (78±3 to 84±5 bpm, $P<0.03$); MAP was unaffected (78±0.8 vs. 77±1.5 mmHg); responses were similar in treatment groups. From 90-120min, C MAP, UBF and UVR were unchanged. E2+TEA (0.8 mM) did not alter MAP from 90-120min, but UBF fell 24±8.9% and UVR rose 38±16% ($P<0.03$, ANOVA). In E2+4AP (0.08 mM) UBF fell 27±5.3%, UVR rose 76±18% and MAP rose 27±6.9% in time-dependent manner ($P\leq0.006$). Uterine cGMP synthesis rose from 108±27 to 386±51 pg/min ($P<0.001$, ANOVA) 120min after E2 and was unaffected by TEA or 4AP. **Conclusions.** UA BK_{Ca} and K_V activation contribute to the maintenance of E2-induced increases in NP UBF, which is partially cGMP-dependent. Uterine and systemic sensitivity to

4AP>>TEA, and 4AP increases MAP >75%, suggesting K_V contribute to blood pressure regulation after E2-induced vasodilation, which may be important in pregnancy. (NIH HD008783)

T-286

Influence of Fetal Sex on Aromatase and Androgen Receptor Expression in the Human Placenta with Preeclampsia. K Sathishkumar, Meena Balakrishnan, Vijayakumar Chinnathambi, Madhu Chauhan, Gary DV Hankins, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*

Numerous studies demonstrate that preeclampsia alters maternal and fetal endocrine profiles including increases in the levels of testosterone and decreases in estrogen levels compared to normal pregnancies. We examined whether preeclampsia affects placental androgen signaling and whether the placenta contributes to some of the increased testosterone levels in preeclamptic pregnancies. Particular attention was paid to determine if the placental expression of androgen receptor and aromatase varied depending on the sex of the fetus.

Study Design: Term placentae (37-41 weeks of gestation) from 11 pregnancies with preeclampsia (5 with female and 6 with male fetus) and 14 normal pregnancies (7 with male and 7 with female fetus) were collected and examined for androgen receptor and aromatase expression by immunofluorescence, Western blotting and quantitative-RT-PCR.

Results: Placental androgen receptor expressions were significantly higher ($P<0.05$) in placentae of both male and female fetus compared to their respective sexes in normal pregnancies. Although the expression of androgen receptor appears to be greater in the male than female preeclamptic placentae, it was not statistically significant. The placental aromatase expression varied depending on the sex of the fetus. If the fetus was female, aromatase levels were substantially higher ($P<0.05$) in preeclamptic than normal placentae. If the fetus was male, the aromatase levels were significantly lower ($P<0.05$) in preeclamptic than normal placentae. Within normal pregnancies, placental aromatase levels were significantly higher ($P<0.05$) in male-than in female-bearing placentae.

Conclusion: These results suggest that the androgen signaling pathways may be over-activated due to overexpressed receptors in the placentae of pregnancies with preeclampsia. In pregnancies affected by preeclampsia, the placenta with male sex contributes, at least in part; to elevated testosterone due to a decrease in aromatase that reduces the conversion of testosterone to estrogens. This fetal sex related differences in placental dysfunctions associated with preeclampsia may differentially impact the male and female fetus that may lead to sexually dimorphic pathophysiological responses during adult life.

T-287

Eicosapentanoic Acid (EPA) Is More Effective Than Docosahexanoic Acid (DHA) in Inhibiting Lipopoylsaccharide-Induced Lipid Hydroperoxide Production in Preterm and Term Placenta. Michael J Stark, Vicki L Clifton, Nicolette A Hodyl. *The Robinson Institute, University of Adelaide, Adelaide, South Australia, Australia.*

Background: Dietary supplementation with ω-3 fatty acids may reduce late preterm birth. However, the relative potency of the ω-3 fatty acids, EPA and DHA, and their mode of action across gestation remains unanswered. With the placenta a major source of inflammatory mediators and oxidative stress implicated in poor pregnancy outcome, we investigated the effects of DHA and EPA on oxidative stress and cytokine production induced by lipo-polysaccharide (LPS) in term and preterm placental explants.

Method: Non-labour term (n=8) and preterm placental explants (n=8) were pre-treated with DHA or EPA (10mM) prior to LPS (1ng) or co-exposed with LPS. Oxidative stress (malondialdehyde - MDA) and pro-inflammatory cytokine production (TNFα, IL6, IFNγ) was measured by ELISA.

Results: EPA and DHA increased MDA compared to controls in term and preterm explants. This increase was lower than that induced by LPS for EPA but not DHA and only in term tissue ($p<0.01$). In term explants, co-treatment with EPA+LPS decreased MDA ($p=0.01$), an effect not observed with DHA+LPS. No effect for EPA or DHA co-treatment was seen in preterm explants. Pre-treatment with EPA or DHA inhibited LPS induced MDA production in term explants ($p=0.01$), an effect also observed for EPA in preterm explants ($p<0.05$). While the oberved effects were not related by alterations in TNFα or IL6 production, EPA/DHA pre-treatment was associated with lower IFNγ production in both term and preterm explants ($p=0.01$).

Conclusions: With current ω3-fatty acid supplementation in pregnancy predominantly DHA, our observation of differential actions in the placenta, effects further influenced by gestation, highlights the need to fully elucidate the mechanisms of action of ω3-fatty acids across gestation.

T-288

Sexual Dimorphic Effects of Moderate Global Maternal Nutrient Restriction (MNR) on Baboon Placental Sp1 Expression. Jaehyek Choi, Chuming Guo, Kang Sun, Peter W Nathanielsz. *OB/GYN, UTHSCSA.*

Introduction: The placenta provides an incomplete barrier to maternal to fetal cortisol transfer. We have shown elevated maternal and fetal cortisol in our baboon model of MNR. In addition placental 11β-HSD 2 expression was reduced at 0.9 gestation (G) in pregnancies with female fetuses. 11β-HSD 2 regulates placental conversion of active cortisol to cortisone. Sp1 is a major transcriptional 11β-HSD 2 regulator.

Hypothesis: Female fetus specific MNR induced decreases in 11β-HSD 2 result from decreased SP-1 synthesis.

Methods: Pregnant baboons were fed ad lib, controls (CTR; n=8) or 70% of CTR global diet (MNR; n= 6) from 0.16 G with fetal euthanasia and tissue recovery under general anesthesia at 0.9 G - Term - 184 days. Protein expression was determined by immunohistochemistry (IHC) and quantified by image analysis for fraction (area immunostained/area of the field x 100%) and density (expressed in arbitrary density units, ADU). Statistics Student's t-test with $p< 0.05$. Data M SEM, CTR presented first, * $p< 0.05$.

Results: A female fetus reduced placental SP-1 protein (Fig 1); mRNA was not different (data not shown). There was good correlation between SP-1 and 11β-HSD 2 protein across all fetuses.

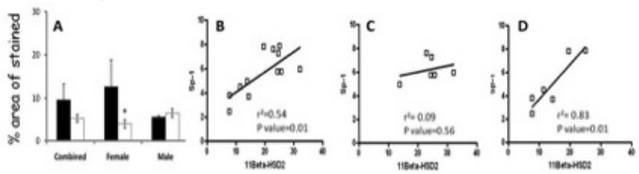


Fig 1. A) Sp-1 placental IHC (fraction) from mothers fed ad lib (CTR; close field: - female fetuses n = 4, male n = 4) or MNR (open field: female n = 3, male n = 3). Regression between 11β-HSD2 and SP-1 B) all pregnancies C) male and D) female fetuses. Data M + SEM; * $p < 0.05$.

Conclusions: SP-1 and 11β-HSD2 protein levels were highly correlated indicating the MNR induced alterations in SP-1 a known regulator of 11β-HSD2 play a role in the down regulation of 11β-HSD2 in female placentas as we hypothesized. These changes would expose female fetuses to higher levels of cortisol than males. Since fetal exposure to cortisol at levels higher than appropriate for the current stage of maturation have been shown to be one of the common elements in developmental programming these observations may partially explain sex of offspring differences in developmental programming outcomes.

T-289

Insulin Resistance as an Etiopathogenetic Factor in Endometrial Cancer: Anatomopathological Correlations with Polycystic Ovary Syndrome. Angela Sacchinelli, Rita Mocciaro, Roberta Venturella, Michele Morelli, Zullo Fulvio. *Obstetrics and Gynecology, University "Magna Graecia," Catanzaro.*

Background: Glucose transporter 4 (GLUT4) appears to be involved in the mechanism of insulin resistance (IR) in women with polycystic ovary syndrome (PCOS). Particularly, a significant reduction of GLUT4 expression in endometrial tissue of these patients has been demonstrated. Although epidemiological studies have shown that obese patients with IR have an increased risk of endometrial cancer, reliable data are not available about this risk in women with PCOS. The aim of this study was to confirm the hypothesis that the IR mediated by, or related to the GLUT4 levels reduction in subjects with PCOS may be an etiological link between hyperplasia and/or endometrial cancer and PCOS.

Design: Observational study.

Patient(s): Twenty-three patients with premalignant endometrial disease (hyperplasia typical, atypical or complex), 25 with malignant disease (endometrial cancer) and 19 PCOS patients not undergone any hormonal or insulin-sensitizing treatment were enrolled as cases. Other 20 healthy women, matched for age and BMI with PCOS population, were enrolled as control group.

Intervention(s): Analysis of GLUT4 mRNA reaction in the endometrial tissue was performed by RT-PCR and immunostaining.

Main Outcome Measure(s): Primary end-point was the GLUT4 expression in pathological endometrium (hyperplasia and endometrial cancer) as a marker of potential indicator of IR. Secondary end-point was the GLUT4 expression in the endometrium of PCOS patients and healthy women.

Result(s): GLUT4 expression was lower in patients with endometrial hyperplasia and endometrial cancer than in PCOS patients and healthy controls. Conversely, in PCOS patients GLUT4 levels were higher than in patients with endometrial hyperplasia and/or endometrial cancer but lower than in healthy controls.
Conclusion(s): The reduced expression of GLUT4 in patients with endometrial hyperplasia and cancer and, even if in a lesser extent, in patients with PCOS comparing to healthy controls, may be an etiological link between hyperplasia and/or endometrial cancer and PCOS.

T-290

Effect of Early Progesterone Supplementation on Adverse Pregnancy Outcomes in PCOS. William M Curtin,[1] Rupali Singh,[2] Anna Lisa Schmitz,[2] Shahab Minassian.[3] *[1]MFM, Ob/Gyn, Penn State College of Medicine, Hershey, PA, USA; [2]Ob/Gyn, The Reading Hospital & Medical Center, West Reading, PA, USA; [3]REI/Ob/Gyn, The Reading Hospital & Medical Center, West Reading, PA, USA.*
OBJECTIVE: Polycystic ovarian syndrome (PCOS) has been associated with adverse pregnancy outcomes. We hypothesized that progesterone supplemented PCOS patients would have a lower incidence of adverse pregnancy outcomes.
STUDY DESIGN: Retrospective cohort study of PCOS patients from a private reproductive endocrinology and infertility practice: subjects were identified from a 10 year span by searching a database for the diagnosis of PCOS and/or infertility. The Rotterdam ESHRE/ASRM criteria for the diagnosis of PCOS were used to identify potential study subjects. PCOS subjects achieving a singleton pregnancy ≥ 20 weeks gestation were selected. Subjects were divided into progesterone supplemented (luteal phase and/or first trimester) and unsupplemented cohorts. Adverse pregnancy outcomes were defined as preterm birth, preeclampsia, gestational hypertension, stillbirth, placental abruption, or fetal growth restriction. Composite adverse outcomes were compared between cohorts with the Chi-square test. We calculated a 24% prevalence of adverse outcomes in the unsupplemented group with a hypothesized reduction of 50% in the supplemented cohort, a power of 80%, and $\alpha = 0.05$ would require 126 subjects in each cohort.
RESULTS: We identified 38 progesterone supplemented and 69 unsupplemented PCOS subjects with singleton pregnancies out of an estimated 400 outpatient charts. The supplemented cohort was slightly older, had a higher rate of ovulation induction and prior spontaneous abortion ($p < 0.05$), while maternal BMI and parity were not different. Comparison of adverse outcomes is given in the table.

Progesterone Supplementation and Outcomes

Adverse Outcome	Supplemented (n=38)	Not Supplemented (n=69)	Odds Ratio (95% CI)
Composite	4 (10.5%)	14 (20.3%)	0.46 (0.12, 1.68)
Preeclampsia	1	9	
Preterm birth	4	6	
Birthweight<10th percentile	0	3	
Gestational hypertension	1	1	
Abruption	0	1	
Stillbirth	1	0	

CONCLUSION: Early progesterone supplementation in PCOS may reduce adverse pregnancy outcomes and is worthy of further study with sufficient numbers of subjects. Theoretically, luteal phase and first trimester progesterone supplementation may enhance the quality of placental implantation and reduce adverse pregnancy outcomes. It may be applicable to other high risk groups.

T-291

Induction of Hyperandrogenism in Lean Reproductive-Age Women Alters the Mononuclear Cell (MNC) Interleuken-1β (IL-1β)Response to Glucose Ingestion in the Presence and Absence of Lipopolysaccharide (LPS). Frank Gonzalez,[1] Janice K Daniels,[2] Dawn M Bearson,[2] Hilary E Blair.[2] *[1]Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA; [2]Obstetrics and Gynecology, and Laboratory Medicine and Pathology, College of Medicine, Mayo Clinic, Rochester, MN, USA.*
Objective: In PCOS, MNC are pre-activated as evidenced by increased cytokine release following glucose or LPS stimulation in the fasting state. We have shown that this proinflammatory phenomenon directly correlates with circulating androgens. We examined the effect of oral androgen administration and LPS exposure on MNC-derived IL-1β release in the fasting state, and in response to glucose ingestion in lean ovulatory women.
Methods: Sixteen lean ovulatory women between ages 18-40 without evidence of androgen excess underwent a 2-hour glucose tolerance test before and after orally ingesting 130 mg of DHEA (n=8) or placebo (n=8) for 5 days, in a randomized double-blind fashion. IL-1β release was measured by ELISA from MNC isolated from blood samples drawn fasting and 2 hours after glucose ingestion, and cultured in the presence and absence of LPS.
Results: Before treatment, subjects receiving DHEA or placebo exhibited no significant differences in body composition, androgens, and IL-1β release from MNC before and after glucose ingestion. Compared to placebo, DHEA treatment raised levels of testosterone (123±9 vs. 45±4 ng/dl, $p<0.0001$) and androstenedione (2.2±0.1 vs. 1.5±0.1 ng/ml, $p<0.002$). After DHEA treatment, the % change in IL-1β release from MNC cultured without LPS increased compared to placebo in the fasting state (34±16 vs. -16±11, $p<0.03$), and in response to glucose ingestion (20±9 vs. -17±15, $p<0.04$). In contrast, the incremental change in IL-1β release from MNC cultured with LPS decreased in the fasting state (-157±63 vs. 113±73 pg/ml, $p<0.02$), and increased in response to glucose ingestion (70±32 vs. -38±31 pg/ml, $p<0.03$) after DHEA treatment compared to placebo.
Conclusion: IL-1β release from MNC of lean reproductive-age women increases after raising circulating androgen to levels observed in PCOS. Addition of LPS suppresses the response to glucose or androgen alone, but not the response to successive exposure to both stimuli. Thus, hyperandrogenemia is proinflammatory in this population due to its ability to induce LPS tolerance, and activate MNC to increase their sensitivity to hyperglycemia.
Supported by NIH grant HD048535.

T-292

Suppression of Lipopoysaccharide (LPS)-Stimulated Cytokine Release from Mononuclear Cells (MNC) of Lean Reproductive-Age Women by *In Vitro* Androgen Exposure – An LPS Tolerance Phenomenon. Frank Gonzalez,[1] Janice K Daniels,[2] Dawn M Bearson,[2] Hilary E Blair.[2] *[1]Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA; [2]Obstetrics and Gynecology, and Laboratory Medicine and Pathology, College of Medicine, Mayo Clinic, Rochester, MN, USA.*
Objective: In PCOS, MNC are pre-activated as evidenced by increased LPS-stimulated cytokine release in the fasting state that directly correlates with circulating androgens. In contrast, this cytokine response is suppressed in lean ovulatory women following androgen administration to raise circulating levels to those observed in PCOS. We examined the effect of *in vitro* androgen exposure on the LPS-stimulated release of TNFα and IL-1β in the fasting state in lean ovulatory women.
Methods: Fasting blood samples were drawn for MNC isolation from 14 lean ovulatory women between ages 18-40 without evidence of androgen excess. MNC were exposed in culture to increasing concentrations of DHEA or testosterone followed by the addition of LPS to the culture medium. Concentrations of TNFα in response to DHEA exposure, and IL-1β in response to testosterone exposure were measured by ELISA in MNC supernatants.
Results: Compared to LPS-stimulated release of TNFα and IL-1β following MNC exposure to respective baseline concentrations of DHEA (175 ng/dl) or testosterone (50 ng/dl), the incremental change in TNFα progressively declined following MNC exposure to DHEA concentrations of 875 ng/dl (-12±9 pg/ml; $p<0.05$), and 1750 ng/dl (-25±10 pg/ml; $p<0.03$); and the incremental change in IL-1β progressively declined following MNC exposure to testosterone concentrations of 125 ng/dl (-24±9 pg/ml; $p<0.03$), and 250 ng/dl (-30±10 pg/ml; $p<0.007$). There was a positive correlation between the responses of TNFα and IL-1β during exposure to increasing androgen concentrations (DHEA 175 ng/dl vs. testosterone 50 ng/dl: $r=0.49$, $p<0.05$; DHEA 875 ng/dl vs. testosterone 125 ng/dl: $r=0.58$, $p<0.02$; DHEA 1750 ng/dl vs. testosterone 250 ng/dl: $r=0.55$, $p<0.03$).
Conclusion: These preliminary data indicate that direct *in vitro* exposure to androgens at concentrations observed in PCOS, or in the presence of an androgen producing tumor suppresses LPS-stimulated cytokine release from MNC of lean reproductive-age women in the fasting state. Thus, increased androgen exposure in this population is capable of inducing LPS tolerance, a phenomenon observed in many inflammatory states.
Supported by NIH grant HD048535.

T-293

The Increased Prevalence of Regular Cycles in Women with Polycystic Ovary Syndrome (PCOS) with Aging Is Associated with Lower Levels of Serum Anti-Mullerian Hormone (AMH). Daniel H Kort,[1] Enrico Carmina,[2] Anna Maria Campagna,[2] Pasquale Mansueto,[3] Roger A Lobo.[1] *[1]Department of Obstetrics and Gynecology, Columbia University, New York, NY, USA; [2]Department of Medical and Biological Sciences, University of Palermo, Palermo, Italy; [3]Department of Clinical Medicine, University of Palermo, Palermo, Italy.*

Several cross sectional studies have shown that the clinical and biochemical presentation of PCOS may attenuate with the natural aging process. However this has not been shown prospectively with a cohort of PCOS patients over time. One hundred and ninety-three women with PCOS of varying phenotypes (Rotterdam criteria) were followed from the time of diagnosis (age 21.9 +/-2.1) for 20 years (age 42.8 +/- 1.5), with the following assessments carried out at 5 year intervals: BMI, waist circumference, ovarian volume, LH, FSH, total Testosterone, DHEAS, Insulin and QUICKI.

Total Testosterone and DHEAS decreased over time, beginning at 10 years and continuing to decrease over 20 years ($p<0.01$). While ovarian volume decreased over the 20 year period ($p<0.01$), waist circumference progressively increased ($p<0.01$). BMI, Insulin, Insulin resistance (QUICKI), and the LH/FSH ratio did not significantly change over the 20 year period. Among patients with anovulatory phenotypes (n=142), 23% (n=33) became ovulatory after 20 years of observation. AMH levels were obtained in a subset of the cohort (n=56) during the 15 and 20 year follow-up visits. Anovulatory patients who became ovulatory (n=12) during this interval had lower baseline AMH levels (4.8 ng/ml +/- 2) vs. those who remained anovulatory. (n= 44) (9.8 ng/ml +/- 4, $p<0.01$) These patients also had lower endpoint AMH levels (2.1 ng/ml +/- 1) vs. those who remained anovulatory. (4.3 +/- 1.7 ng/ml, $p<0.01$)

Conclusion: In women with PCOS, there is a gradual decline in serum androgens and ovarian volume over time. While certain metabolic factors remain unchanged, waist circumference increases. Although AMH is increased in all women with PCOS, women with relatively lower levels appear to be those most likely to become ovulatory with normal menstrual function as they age.

T-294

Thyroid Dysfunction in a Large Cohort of Normogonadotropic Normoestrogenic Women and Women with Polycystic Ovary Syndrome. Yvonne V Louwers,[1] Anne van Zessen,[1] Myrthe Bandell,[1] Erwin Birnie,[1,2] Diana BCM Dufour-van den Goorbergh,[3] Herbert Hooijkaas,[3] Theo J Visser,[4] Joop SE Laven.[1] *[1]Division of Reproductive Medicine, Department of Obstetrics and Gynecology, ErasmusMC University Medical Center, Rotterdam, Netherlands; [2]Department of Health Policy and Management, ErasmusMC University Medical Center, Rotterdam, Netherlands; [3]Department of Immunology, ErasmusMC University Medical Center, Rotterdam, Netherlands; [4]Department of Internal Medicine, ErasmusMC University Medical Center, Rotterdam, Netherlands.*

Background Anovulation can be the result of thyroid dysfunction which interferes with numerous aspects of reproduction. Prevalence of auto-immune thyroid disease is suggested to be higher in infertile women as well as in women with polycystic ovary syndrome (PCOS).

Objective Our aim was to assess thyroid function based on serum levels of thyroid stimulating hormone (TSH), free thyroxin (FT4) and thyroid peroxidise auto antibodies (TPOAb) in a large cohort of normogonadotropic normoestrogenic women and PCOS women.

Methods Based on diagnostic criteria recommended by the World Health Organization (WHO) women presenting with anovulation and serum follicle stimulating hormone (FSH) and estradiol (E2) levels within the normal range were classified as WHO group 2 (WHO2). PCOS was diagnosed according to the Rotterdam criteria. Healthy controls were derived from the Rotterdam Blood Bank. Serum levels of FT4, TSH and TPOAb were determined in all women. Analysis of variance (ANOVA) adjusted for differences in BMI and age was applied to determine differences between groups. $P<0.05$ was considered to be statistically significant.

Results A total of 1603 WHO2 women were included, of which 1450 women were classified as PCOS. In 58/1603 (3.6%) WHO2 women serum TSH levels were elevated and TPOAb were positive in 91/1603 (5.7%) of the WHO2 women. In PCOS women 81/1450 (5.6%) showed elevated serum TSH levels whereas elevated TPOAb were found in 81/1450 (5.6%) of the PCOS women. In all women serum FT4 levels were within normal range. Compared to healthy controls serum TSH levels and TPOab were not elevated in WHO2 women or in PCOS women.

Conclusion Although literature in smaller sized study populations suggests that anovulatory women have a higher chance of developing thyroid disease, we could not confirm these findings in our large study population. A greater risk of developing thyroid disease in the future is not to be expected in these women.

T-295

Standardized Mortality Rate in Parents of Women with the Polycystic Ovary Syndrome (PCOS). Yvonne V Louwers,[1] Marieke Roest-Schalken,[1] Jeanine Roeters-van Lennep,[2] Eric J Sijbrands,[2] Joop SE Laven.[1] *[1]Division of Reproductive Medicine, Department of Obstetrics and Gynecology, ErasmusMC University Medical Center, Rotterdam, Netherlands; [2]Department of Internal Medicine, ErasmusMC University Medical Center, Rotterdam, Netherlands.*

Background The polycystic ovary syndrome (PCOS) is associated with multiple risk factors of the metabolic syndrome as well as cardiovascular disease (CVD) and could therefore influence life expectancy. Because of the familial nature of the syndrome, parents of PCOS women are also prone to develop these risk factors. A lot is known on children of PCOS women but data on the parents of PCOS women is scarce. A recent study found that fathers of PCOS women had a higher prevalence of heart attack and stroke compared to a reference population. Thus far data on mortality rates in parents of PCOS women is lacking.

Objective This study determines all-cause mortality in parents of PCOS women using Standard Mortality Rate analysis (SMR).

Methods PCOS was diagnosed according to the Rotterdam criteria. Parental information was obtained during initial screening of the PCOS women and questionnaires were sent to update the medical history of the parents. Additional data on date of birth and death of the parents of the PCOS women was achieved from a nationwide municipal records web-based database. SMR was calculated based on the mortality of the parents of PCOS women compared to the general population standardized for age, gender and historical mortality rates. Furthermore subgroups were selected based on the presence of specific health risks such as cardiovascular disease and diabetes.

Results Overall life expectancy in the parents was not influenced by the PCOS of their daughters. However, a significant decrease in life expectancy in the mothers with diabetes mellitus was observed (SMR 1.81; p-value=0.02). Fathers with diabetes (SMR 1.12; p-value=0.34) and parents known with CVD (fathers SMR 1.13, p-value=0.20; mothers SMR 1.3, p-value=0.11) had a shorter life expectancy. These differences are not significant but tend to strike in the unfavorable direction.

Conclusion This is the first study to assess mortality rate in parents of PCOS women. Mortality rates these parents were similar to the general population. However, the diabetic mothers of PCOS patients did have a significantly shorter life expectancy. Development of diabetes in mothers of PCOS women might identify a high risk group who deserve special attention in screening programs.

T-296

The Association between PCOS and Psoriasis: Results from a Pilot Study. Francesca Moro,[1] Daniela Martinez,[1] Anna Tropea,[1] Maria Francesca Gangale,[1] Francesca Sagnella,[1] Andrea Morciano,[1] Carola Palla,[1] Andrea Ciardulli,[1] Maria Letizia Uras,[1] Elisa Scarinci,[1] Angela Teti,[1] Antonio Lanzone,[2] Rosanna Apa.[1] *[1]Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, Roma, Italy; [2]OASI Institute for Research, OASI Institute for Research, Troina, Italy.*

Polycystic ovary syndrome (PCOS) is a frequent condition, affecting about 10% of fertile women. Insulin resistance plays a role in the pathogenesis of PCOS and is associated with a high risk of cardio-metabolic abnormalities. Moreover, women with PCOS seems to have a pro-inflammatory state and an increased prevalence of non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome. All these pathological conditions are also been demonstrated in patients affected by psoriasis. The aim of this case-control study was to evaluate a possible association between psoriasis and PCOS. 98 women with psoriasis and 97 controls, aged 14-35 years, were enrolled. PCOS diagnosis was based on the Rotterdam criteria. At day 3 of a spontaneous or induced (with medroxyprogesterone acetate, 10 mg/day for 7 days) menstrual cycle all patients underwent a clinical examination, which included measurements of blood pressure, BMI, and waist-hip ratio (WHR) as well as transvaginal ultrasonography. On the same day, testosterone (T), sex hormone–binding globulin (SHBG), free androgen index (FAI), androstenedione (A), 17-hydroxyprogesterone (17-OHP), dehydroepiandrosterone sulphate (DHEAS), triglycerides, total cholesterol, high- and low-density lipoprotein cholesterol (HDL and LDL), and complete blood count were assayed. In our population PCOS was diagnosed in 11,1 % of control patients and in 47,1% of

patients affected by psoriasis. Furthetmore psoriasis was strongly associated with PCOS risk (OR:4.2, 95% C.I.: 1.04-17.18, p:0.027). In conclusion we have identified a strong association between Psoriasis and PCOS. These results, if confirmed in other cohorts, may introduce a new approach to the prevention of metabolic and cardiovascular risk in patients with psoriasis.

T-297

Effects of Metformin-Oral Contraceptive Combined Therapy on CD4+CD28 Null T Lymphocytes Frequency in Hyperinsulinemic PCOS Women. Francesca Sagnella,[1] Anna Tropea,[1] Maria Francesca Gangale,[1] Francesca Moro,[1] Daniela Martinez,[1] Andrea Morciano,[1] Carola Palla,[1] Andrea Ciardulli,[1] Maria Letizia Uras,[1] Elisa Scarinci,[1] Angela Teti,[1] Antonio Lanzone,[2] Rosanna Apa.[1] *[1]Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, Roma, Italy; [2]OASI Institute for Research, OASI Institute for Research, Troina, Italy.*

Women with polycystic ovary syndrome (PCOS) have an increased risk of cardiovascular disease. Our recent study demonstrated an expansion of CD4(+) CD28(null) T cells in PCOS women. These cells represent an aggressive population of T lymphocytes that has been recently associated with recurrent coronary instability and type 2 diabetes mellitus. The aim of the present study was to evaluate the effects of metformin versus oral contraceptives (OC) versus combined metformin-OC therapy on CD4+CD28 null T cells frequency, hyperinsulinemic PCOS women. 60 PCOS women were enrolled and randomized to receive an OC containing ethinilestradiol 30 µg–drospirenone 3 mg (20 patients) or metformin 1500 mg daily (20 patients) or a combination of these two drugs (20 patients). At day 3 of a spontaneous or induced (with medroxyprogesterone acetate, 10 mg/day for 7 days) menstrual cycle, at baseline and after four mounts of treatment, all patients underwent a clinical examination, a transvaginal ultrasonography and a hormonal and biochemical evaluation. A venous blood sample was also performed to analyze both total blood cells count and CD4+CD28 null T cells frequency. After four months of treatment, metformin-OC combined therapy is able to significantly reduce the expression of CD4+CD28 null T lymphocytes in PCOS patients (*median 4.04 vs 2.03; p=0.002*). Therapy with metformin alone seems to induce a trend in reduction of this lymphocyte subpopulation (*median 3.3 vs 2.8; p=0.09*). No statistically significant difference was observed after treatment with OC. In conclusion our results demonstrated that, in PCOS patients, metformin-OC combined therapy decreases the expression of CD4+CD28 null T lymphocytes. These data appear interesting considering the increased cardiovascular risk in young PCOS patients.

T-298

Increased Insulin-Resistance and β-Cell Function in Obese Polycystic Ovary Syndrome Women. Ido Sirota, Daniel E Stein. *Department of Obstetrics and Gynecology, St. Luke's Roosevelt Hospital, New York, NY, USA.*

Objective:

To compare insulin-resistance and pancreatic β-cell function of obese and non-obese women with polycystic ovary syndrome (PCOS).

Methods:

In a retrospective study conducted on fifty-one women with PCOS of reproductive age, demographic and laboratory data were abstracted from our computerized database. Body Mass Index (BMI) was stratified as: healthy weight (BMI 18.5 - 24.9 kg/m²), overweight (BMI 25 - 29.9 kg/m²) and obese (BMI ≥ 30 kg/m²). PCOS was diagnosed by the Rotterdam criteria 2003 consensus workshop. Insulin resistance and β-cell function were measured using the Homeostatic Model Assessment Insulin Resistance (HOMA-IR) and β-cell function (HOMA- β%). HOMA-IR and HOMA- β% were compared among the three BMI groups. Statistical analyses were performed using the SPSS 15.0 software for Windows®. Quantitative variables were compared by one-way ANOVA (data with normal distribution). The level of significance was set at 5% ($p<0.05$) in all analyses.

Results:

51 patients participated in the study: 57% (29/51) healthy weight, 21.5% (11/51) overweight, 21.5% (11/51) obese. HOMA-IR was significantly higher in obese women in comparison to overweight and healthy weight patients, (mean ± S.D. : 2.88±2.09, 1.13±0.73, 0.84±0.49, respectively, $p<0.000$). Moreover, HOMA- β% was significantly increased in obese women in comparison to the other two groups, (mean ± S.D. : 186.89±131.62, 106.83±46.77, 86.60±40.91, respectively, $p<0.001$). In contrast, no difference was found between healthy weight and overweight subjects.

Conclusions:

Our data demonstrates higher insulin resistance and β-cell function in obese PCOS patients as compared to non-obese PCOS patients using the HOMA-IR and HOMA- β% calculations. In contrast, no differences in these parameters were observed between healthy weight and overweight PCOS patients.

T-299

Chew on This: Knowledge of the Effects of Obesity on Reproductive Outcomes in an Urban Community. Eden R Cardozo,[1] Tanaka J Dune,[1] Maureen E Brocks,[1] Geraldine E Ekpo,[1] Lisa M Neff,[2] Randall B Barnes,[1] Erica E Marsh.[1] *[1]Obstetrics and Gynecology, Feinberg School of Medicine - Northwestern University, Chicago, IL, USA; [2]Medicine; Division of Endocrinology, Feinberg School of Medicine - Northwestern University, Chicago, IL, USA.*

Background: Nearly 65% of women in the United States are overweight/obese, and obesity is a significant risk factor for adverse reproductive health outcomes. No published studies have assessed public awareness of these risks.

Objective: This study seeks to explore knowledge of obesity as a risk factor for reproductive and general health problems, and to determine what demographic factors correlate with body weight knowledge in an urban community.

Design: Cross-sectional survey study.

Methods: A convenience sample of English-speaking men and women over age 18 who attended an urban community fair in a large midwestern city were recruited. After informed consent was obtained, participants completed a questionnaire on the health risks of obesity.

Results: 303 adults participated in the study. 76.9% were female and 98.3% were black. Average age was 50.6 for men and 51.4 for women. Average BMI was 28.8 for men and 30.7 for women. 73.3% had at least some college education. 66.5% had a household income of less than or equal to $50,000. 84.9% had a primary care doctor, and 84.1% had been seen by a doctor in the past year. 73.2% of subjects had heard of the term BMI, but only 20.9% thought they knew their BMI and only 7.1% correctly knew their BMI. 18.2% of subjects knew the ideal BMI range. 78.3%, 73.2%, and 75.5% of subjects knew that being overweight/obese increased one's risk of diabetes, heart disease and hypertension respectively. Few subjects knew that increased weight is associated with increased risk of miscarriage (30.8% men, 38.5% women), irregular periods (22.9% men, 35.7% women), infertility (21.3% men, 33.0% women), c-section (14.3% men, 31.7% women), birth defects (22.9% men, 24.6% women), endometrial cancer (12.2% men, 18.0% women), and stillbirth (10.4% men, 13.9% women).

Conclusions: The majority of men and women at an urban community fair were unaware of the reproductive risks associated with obesity. Most, however, had knowledge of the non-reproductive health effects of obesity. Most subjects did not know their own BMI or the normal range for BMI. Health education for the general population is needed on the effects of obesity on reproductive health outcomes.

T-300

Enrollment in a Tobacco Smoking Cessation Program and Regression of Abnormal Cervical Cytology. Diana P English,[1] Fausto Andrade,[1] Monica Pasternak,[1] Asma Aftab,[2] Jorge J Garcia.[1] *[1]Obstetrics and Gynecology, University of Miami/Jackson Memorial Hospital, Miami, FL, USA; [2]Family Medicine and Community Health, University of Miami/Jackson Memorial Hospital, Miami, FL, USA.*

Introduction

A consistent association between cigarette smoking and cervical dysplasia has been noted in previous studies. We sought to assess the relationship between smoking cessation, as achieved through a dedicated smoking cessation program at our County Hospital and the spontaneous regression of cervical precursor lesions.

Methods: This is a pilot study. Records were reviewed of smokers that attended our colposcopy clinic between January, 2006 and July, 2011. All smokers were referred to our smoking cessation program. The patients who enrolled in this program were then compared with those who continued their smoking habits. The smoking cessation database was also queried in order to identify interventions received and patient compliance. The triage of abnormal pap smears at presentation and follow-up was based on the guidelines by the ASCCP.

Results

We identified 32 smokers. 18 patients enrolled in the smoking cessation program and 14 patients declined. There were no statistical differences in the age, ethnicity, High-risk HPV presence, BMI and presence of STD's between the two groups. The mean duration until the return to negative cervical cytology

in those enrolled in the program was 18.5 months compared to 46.9 months in those who declined. There was a significant trend towards an association between enrollment in smoking cessation and return to a negative pap smear with a p-value of 0.07.

	Enrolled	Not Enrolled	P-value
Mean duration of smoking (yrs)	16.6	11.4	0.14
Mean duration of enrollment(mths)	11.7	----------	
% HR-HPV	33.3	57.1	0.28
% HIV (+)	50	14.3	0.06
Mean age (yrs)	44	38	0.18
% Other STD's	16.6	0	0.24
% Ethnicity			
African-American	50	21.4	0.15
White	16.6	21.4	1
Hispanic	33.3	57.1	0.29
% Initial degree of dysplasia			
ASCUS	50	42.9	0.73
LGSIL	33.3	42.9	0.72
HGSIL	11.1	14.3	1
Mean duration to negative cytology (mths)	18.5	46.9	0.07

Abnormal Cervical Cytology in Smokers

Conclusion

There was a significant trend towards an association between enrollment in smoking cessation and quicker return to a negative pap smear. Although not statistically significant, largely due to a small sample size, this study has provided the preliminary data to support a larger study evaluating the role of a dedicated smoking cessation in ameliorating abnormal cervical cytology.

T-301

Characteristics of Women with Late Antenatal Booking in the Netherlands. Kirsten M Heetkamp,[1,2] Rachel Bakker,[2] Hanneke W Torij,[1] Eric AP Steegers,[2] Gouke J Bonsel,[2] Semiha Denktas.[2] *[1]Centre of Expertise, Innovations and Care, Rotterdam University of Applied Science, Rotterdam, Netherlands; [2]Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, Netherlands.*

Objective To study the characteristics of women with late first antenatal booking (gestational age ≥12 weeks) including age, parity, ethnicity and socio-economic status(SES).

Methods Data from single pregnancies in the period 2000-2007 were derived from the Dutch Perinatal Registry. This registry contains population-based information of 97% of all pregnancies in the Netherlands. Logistic regression analysis was used to study the associations between characteristics of women and late first antenatal booking.

Results The overall percentage of women with first antenatal booking at 12 weeks or later was 47.4% (n=502086). A decrease of the percentage of late entry in antenatal care was observed over the years: 58.5% in 2000 to 22.6% in 2007. Among teenagers, women ≥40 years, multiparous, immigrants and women with low SES the highest risks of late entry in antenatal care was observed.

Table 1. Associations of characteristics of women and late first antenatal booking (n=1060149).

	Total n	≥12 wks %	Crude ORs (95%CI)*	Adjusted ORs (95%CI)*
Age				
<20 yrs	17987	70.0	2.78(2.69,2.87)	2.29(2.22,2.37)
20-24 yrs	112160	52.6	1.32(1.30,1.34)	1.13(1.11,1.14)
25-29 yrs	315508	44.0	0.94(0.93,0.94)	0.90(0.89,0.91)
30-34 yrs	424263	45.7	Ref	Ref
35-39 yrs	169934	50.2	1.20(1.19,1.21)	1.14(1.13,1.15)
≥40 yrs	20101	61.9	1.93(1.88,1.99)	1.59(1.55,1.64)
Parity				
P0	501882	46.8	Ref	Ref
P1	379420	45.0	0.93(0.92,0.94)	0.94(0.93,0.95)
P2	127887	50.3	1.15(1.14,1.16)	1.10(1.08,1.12)
P3	33486	59.6	1.68(1.64,1.72)	1.46(1.43,1.49)
P4	10025	68.6	2.49(2.38,2.60)	2.33(2.17,2.51)
P5	3919	72.5	3.00(2.80,3.22)	3.00(2.80,3.22)
≥P6	3530	78.6	4.17(3.85,4.52)	3.17(2.92,3.44)
Ethnicity				
Native Dutch	867710	44.1	Ref	Ref
Immigrant	185898	62.1	2.08(2.06,2.10)	1.91(1.88,1.93)
Socio-economic Status (SES)				
SES <P20	259743	52.6	1.30(1.29,1.32)	1.06(1.05,1.07)
SES P20-P80	616124	46.0	Ref	Ref
SES >P80	183571	44.7	0.95(0.94,0.96)	0.97(0.96,0.98)

* All P-values <0.001

Discussion Almost half of all pregnant women in the Netherlands entry antenatal care after 12 weeks of gestation. We observed the highest risks of late antenatal booking in teenagers, older women (≥40 years), multiparous, immigrants and women with lower SES. Previous studies of Chote et al. (2009) and Alderliesten et al. (2007) found similar results. Future research should study the effect of late first antenatal booking on pregnancy outcomes.

T-302

Completion Rates of the Human Papilloma Virus Vaccine for Insured Females and Males in the United States, 2006-2010. Jacqueline M Hirth,[1] Alai Tan,[2] Gregg S Wilkinson,[2] Abbey B Berenson.[1] *[1]OB/GYN, University of Texas Medical Branch, Galveston, TX, USA; [2]Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, TX, USA.*

Context: Gardasil is a vaccine that protects against four prevalent and high-risk HPV types. Gardasil is administered in 3 doses, and those doses should be administered within one year to provide optimum immunogenic protection in all recipients.

Objective: To determine the effects of age, gender, time, and type of health provider on HPV vaccine completion rates.

Design: A retrospective cohort study.

Setting: Secondary data analysis of a national database of medical claims.

Participants: Initiators of the Gardasil vaccine series who were continuously enrolled in their respective insurance plan for 365 days.

Main Outcome Measures: Vaccination was considered complete if there were three vaccine claims from 3 distinctive dates within 365 days in a subject's record. Binary logistic regression determined the odds of completing the series within one year of initiation.

Results: Females were more likely to complete (OR: 3.551, 95% CI: 2.941-4.288) than males. Completion rates declined from 50.6% in 2006 to 21.5% in 2009 among females, and from 16.4% in 2006 to 4.8% in 2009 among males. Subjects 18-26 years (OR: 0.823, 95% CI: 0.807-0.840) and >26 years (OR: 0.490, 95% CI: 0.459-0.523) were less likely than those 9-17 years to complete. Clinics/ other facilities (OR: 0.546, 95% CI: 0.473-0.631), nurses (OR: 0.892, 95% CI: 0.808-0.985), and specialists (OR: 0.828, 95% CI: 0.748-0.917) were less likely than pediatricians to administer completed vaccinations.

Conclusions: Completion rates have decreased dramatically since 2006 among the insured. Steps need to be taken to encourage initiators to complete the vaccination series and to educate physicians who may not be as familiar with the vaccine to emphasize the importance of completing the series to their patients. More research about vaccination completion differences between males and females needs to be done.

T-303

An Update on Human Papillomavirus Vaccine Uptake among 11-17-Year-Old Girls in the United States: National Health Interview Survey, 2010. Tabassum H Laz, Mahbubur Rahman, Abbey B Berenson. *Center for Interdisciplinary Research in Women's Health and Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

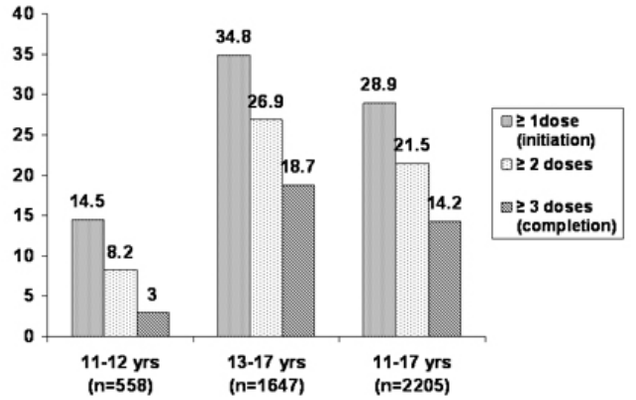
Background: A 3-dose human papillomavirus (HPV) vaccine given over 6 months is recommended for adolescent girls to protect against HPV infections and cervical cancer.

Objective: To provide an update on the HPV vaccine coverage among 11-17 year-old girls based on 2010 data.

Methods: Data from the 2010 National Health Interview Survey were obtained to assess HPV vaccine uptake among girls (11-17 y) and their correlates. Multivariate logistic regression analyses were performed to examine the correlates of initiation (≥1 dose) and completion (≥3 doses) of HPV vaccine.

Results: Overall, 14.5% and 3.0% of 11-12 year-old girls, and 34.8% and 18.7% of 13-17 year-old received ≥1 dose and 3 doses of HPV vaccine, respectively. Compared to whites, Hispanics had a higher rate of initiation (odds ratio (OR) 1.63, 95% confidence interval (CI) 1.22-2.17). Influenza shot in the past year and parental familiarity with HPV vaccine were significantly associated with both initiation (OR 1.86, 95% CI 1.50 -2.31 and OR 16.35, 95% CI 10.82 -24.71) and completion (OR 1.46, 95% CI 1.12 -1.90 and OR 10.39, 95% CI 5.84 -18.49). Among parents who were interested but did not vaccinate their daughters, 53.7% of them were willing to pay the HPV vaccine cost while 41.7% preferred to get low-cost or free vaccine. Three main parental reasons for not being interested in getting their daughters vaccinated were: "does not need vaccine" (25.5%), "worried about safety" (19.3%) and "does not know enough about vaccine" (16.6%).

Conclusion: Nearly one out of three girls aged 11-17 years initiated the vaccine while half of the initiators completed all 3 doses. Health care providers need to educate parents about the importance of vaccination to protect their daughters against HPV infections along with the necessity to complete the series. Measures are also needed to identify unvaccinated populations to encourage HPV vaccination and guide those who are worried about the cost to low-cost or free vaccine programs for this age group.



T-304

Evolving Trends in Maternal Fetal Medicine Referrals in a Rural State: The Arkansas Experience. Everett F Magann,[1] Janet Bronstein,[2] Samantha S McKelvey,[1] Dora M Smith,[1] Paul J Wendel,[1] Curtis C Lowery.[1] *[1]Obstetrics and Gynecology, University of Arkansas for Medical Sciences, LIttle Rock, AR, USA; [2]Department of Health Care Organization, University of Alabama, Birmingham, AL, USA.*

Objective: To determine the trends in maternal fetal medicine (MFM) referrals in a Medicaid population in a rural state over time.

Methods: Sixteen guidelines and 23 clinical conditions were identified where co-management or consultation with MFM specialist is recommended. We used linked Medicaid claims and birth certificate data for 2001-2006 to identify pregnancies with these conditions and assess whether they received care from a maternal fetal medicine specialist. We assessed whether the ANGELS intervention (teleconference rounds, on-line obstetric and neonatal guidelines and a 24-hour MFM consultation service) influenced the likelihood of a MFM consult.

Results: Between 2001 and 2006, there were 108,703 pregnancies with delivery of 110,890 neonates. Forty five percent were identified as having one or more of the conditions listed for co-management/consultation. Pregnancies receiving any MFM contact remained unchanged at 22.2% in 2001 and 22.1% in 2006. However, face to face contacts decreased from 14.7% (2001) to 9.2% (2006) while telemedicine consults increased from 7.6% (2001) to 12.6% (2006). We observed demographic changes in the population and marked changes in prenatal care sources over time which increased the availability of local obstetrics care for Medicaid covered women. Health departments were most likely and family practitioners least likely to refer to MFM specialists ($p< 0.001$). Pregnancy complications most likely to lead to MFM referrals include cardiac complications, renal disease, systemic disorders, PPROM, suspected fetal abnormalities, and cervical insufficiency, with health departments referring for poor obstetric history and hypertension. Providers participating in teleconference rounds and those who used the physician call center were more likely to refer high risk patients, while establishment of consensus practice guidelines reduced MFM referrals from health departments.

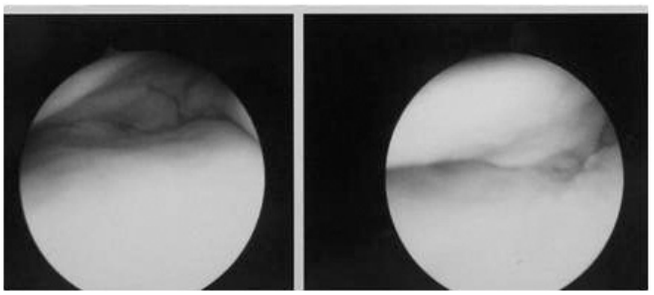
Conclusions: Referral of high risk pregnancies to MFMs varies with the level of expertise available at the primary prenatal care site. Taking this into account, however, increased contact between MFMs and local providers via teleconference rounds and a call center increased the likelihood of MFM referrals for high risk pregnancies.

T-305

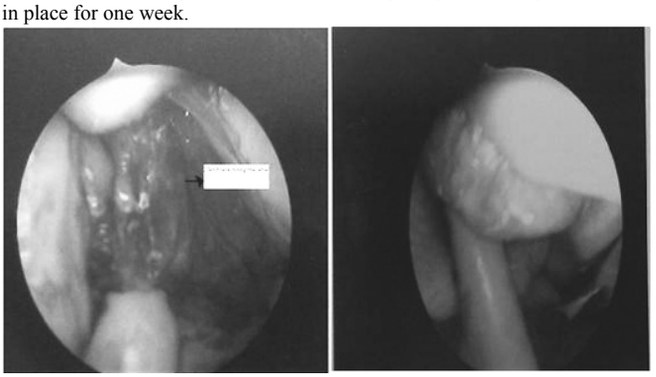
Total Labial Adhesion Obstructing the Urethra – A Case Report. Ismail Mert, David Kmak. *Obstetrics and Gynecology, Wayne State University.*

Introduction: Labial adhesions usually seen in pre-pubertal girls and relatively rare in old women. We present a case with complete labial adhesion which obstructs the urinary outlet.

Case: An 82 year old woman referred to DMC secondary to difficulty in urinating and pelvic pain. Patient has a long history of straining during urination, applying pressure from the supra-pubic region and rectum in order to empty her bladder. Past surgical history was significant for total abdominal hysterectomy and bilateral salphingooeferectomy secondary to fibroids. On examination, both labium majus and minus were fused to each other and urethra cannot be visualized. A decision was made to release the adhesions first. Blunt and sharp dissections of the adhesions were performed. While dissection, an accidental entry into a space occurred and approximately 20 cc urine drained form that space. Rectal injury was ruled out by digital exam. The cavity was further inspected with hysteroscopy and normal vaginal cuff and normal vaginal mucosa were noted.



Urethral meatus was visualized. No fistula noted. At this time, it was realized that, she was draining her bladder into her vagina and then from vaginal space to outside by applying pressure to her rectum from a potential hole on the membrane that fused the labia which was not detected during the surgery. The membrane fusing the labia dissected completely and a foley catheter left in place for one week.



Patient was re-evaluated at the office at the end of the week and noted be without complications with well healing labia

Conclusion: In old woman, labial adhesions can be a source of urethral obstruction.

T-306

Male Offspring (OFF) Exercise (OFFEx) Reverses Dysfunctional Changes in Glucose (GL) and Lipid (LIP) Metabolism Programmed by Maternal Obesity (MO). Luis Reyes,[1] Fabiola Cruz-Perez,[1] Claudia Vega-Garcia,[1] Claudia J Bautista,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] *[1]Departo de Biolgia de la Reproduccion, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; [2]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Human epidemiologic and rodent studies show that OFF exposed to MO in development have altered body composition and predisposition to metabolic disease and obesity (OB). World-wide > 30% of reproductive age women are OB. The current childhood OB epidemic is likely due to exposure to both maternal OB developmental programming and sedentary post-natal (PN) lifestyle plus obesogenic diet. We hypothesized that OFFEx is beneficial in decreasing programmed OFF predisposition to OB and metabolic disease.

METHODS. We fed female Wistar rats from weaning through pregnancy and lactation on chow (C) or high energy obesogenic diet (MO). Mothers were bred at PN day (PND) 120 and diet maintained until OFF weaned to C. Male OFF (n=8 different litters) wheel-ran 30 min, 5 times/ week from PND 50 to 110 (OFFEx) and euthanized at PND 110, fat depots weighed, body weight (BW), food intake (FI), serum leptin, triglycerides (TG), GL, insulin and HOMA measured. Statistics Two way ANOVA (maternal diet and Ex); Data M ± SEM.

RESULTS: OFFEx in C reduced leptin but not fat (Fig 1). OFFEx in MO completely reversed MO induced increases in BW, GL, fat, leptin and partially – TG and HOMA (11.2±1.9 vs, 8.2±1.2 – $p< 0.09$.) There were no differences in distance run (C: 150 m/30 min and MO: 170 m/30 min). Food intake g/ day: C: 26 ± 0.3, CEx: 24±0.3, MO: 27 ± 0.2, OFFEx: 25±0.08), Food intake g/100 g body wt: C: 6 ± 0.1, Cex: 5.8 ± 0.2, MO: 5.8 ± 0.1, OFFEx: 5.8 ± 0.1).

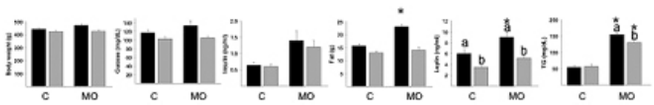


Fig 1. No OFFEx (solid) OFFEX (grey) n=8; M ±SEM. Different letters significantly different OFF vs OFFex, * vs C, $p < 0.05$.

CONCLUSIONS: Regular OFFEx reverses the decreased insulin sensitivity, increased fat and leptin (and TG partially) in MO OFF. These findings reinforce the need for interventions promoting exercise in OFF of OB mothers.

T-307

Female Genital Mutilation/Cutting: An Update. Abdulrahim A Rouzi,[1] Faisal Alturky.[2] *[1]Obstetrics and Gynecology, King Abdulaziz University; [2]Obstetrics and Gynecology, McGill University.*

Objective: To assist Western health care providers in recognizing and addressing the vast medical needs of the women and girls with a history of FGC/M.

Materials and Methods: Data for this review were identified by literature search in the electronic databases MEDLINE and PubMed and the references from relevant articles through August 1, 2011 using the search terms "FGC", "FGM", and "Female Circumcision." Only articles published in English were included.

Results: Studies of FGC/M suffer from many methodological problems including inadequate analysis and an unclear reporting of results. Unnecessary anxiety due to delay in surgical treatment and investigations like comprehensive endocrinology tests, ultrasonography, and MRI are performed to evaluate epidermal clitoral inclusion cysts. The evidence to link FGC/M to infertility is weak. Defibulation is recommended to be performed by a senior person with extensive experience in FGC/M in specialized centers.

Conclusions:

A significant amount of education is required to improve and correct the inadequate care of women who have experienced FGC/M.

T-308

Placental sFlt-1 mRNA Expression Is Significantly Upregulated in Preeclampsia. Aletta Buurma,[1] Marlies Penning,[1] Marie-Louise van der Hoorn,[2] Jan Anthonie Bruijn,[1] Kitty Bloemenkamp,[2] Hans Baelde.[1] *[1]Dept. of Pathology, Leiden University Medical Center, Leiden; [2]Dept. of Obstetrics, Leiden University Medical Center, Leiden.*

Introduction

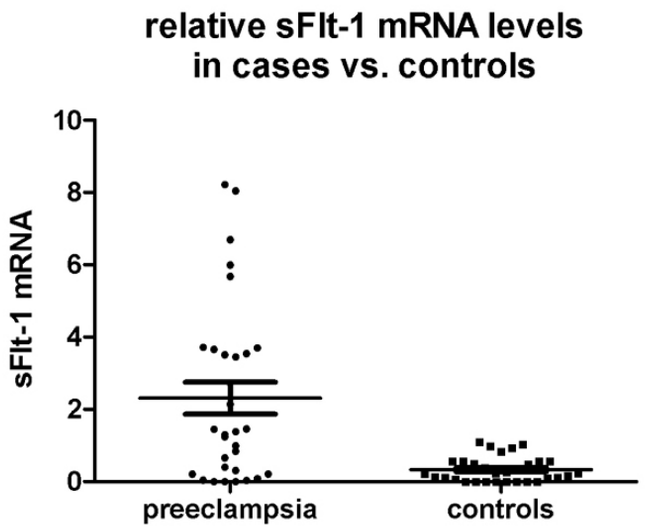
sFlt-1 is one of the most important anti-angiogenic factors that is abundantly present in the circulation of women with preeclampsia (PE). It is believed to be one of the causative factors in the endothelial dysfunction that characterizes PE. Several sources of sFlt-1 have been suggested, including the placenta but also maternal peripheral blood mononuclear cells. Although differences in placental sFlt-1 mRNA levels have been described in previous studies, most of these studies included a limited number of placentas or used cultured cells. This study was set up to measure placental sFlt-1 mRNA production and to determine which cells within the placenta produce sFlt-1.

Methods

Placentas obtained from women with PE (n = 32, defined according to ACOG criteria) were compared to placentas from healthy subjects (n = 37). From these placentas mRNA was isolated, and subsequent quantitative PCR (qPCR) was performed to measure sFlt-1 mRNA expression levels. Additionally, in situ hybridization (ISH) was performed to assess which placental cells produce sFlt-1.

Results

qPCR showed on average a 7-fold increase in placental sFlt-1 mRNA expression when comparing controls to placentas obtained from women with PE ($p<0,01$).



SH for sFlt-1 markedly stained the syncytiotrophoblast. Moreover, several intravillous cells also stained positively for sFlt-1 mRNA, which appeared to be macrophages.

Discussion

Increased sFlt-1 mRNA expression was demonstrated in a substantial number of placentas from women with PE as compared to controls. These results are in accordance with the idea that excessive circulating sFlt-1 during PE is mainly placenta-derived, although an extra-placental source of sFlt-1 cannot be excluded. Which specific cell types are responsible for placental overexpression of sFlt-1 mRNA was assessed by ISH, showing strong staining of the syncytiotrophoblast and also of several intravillous cells. To investigate the nature of these cells, combined ISH and immuno-staining for macrophages will be performed.

T-309

Placental Growth Factor (PlGF) Maternal Circulating Levels in Normal Pregnancies and in Pregnancies at Risk of Developing Placental Insufficiency Complications. S Calabrese, M Cardellicchio, M Mazzocco, E Taricco, A Martinelli, I Cetin. *Dept Ob Gyn, Hospital L.Sacco, Center for Fetal Research Giorgio Pardi, Univeristy of Milan, Italy.*

OBJECTIVES: Low levels of pro-angiogenic PlGF are involved in the patho-physiology of preeclampsia (PE). Contrasting data have emerged for the role of PlGF in the pathogenesis of intrauterine growth restriction (IUGR). The aim of our study was to investigate prospectively PlGF levels in normal pregnancies and in pregnancies at risk of developing complications related to placental insufficiency.

METHODS: Circulating PlGF levels were measured by the Triage® PlGF Test (Alere). Ninety-one normal pregnancies were sampled in the 1st, 2nd and 3rd trimester. A further 39 pregnancies "at risk" of developing placental insufficiency complications (N=16) or at onset of PE (N=15) or IUGR (N=8) were sampled between 19 and 35 weeks to evaluate utility of PlGF. Patients at risk were identified at the first obstetric examination, when a risk score was attributed to the mother, based on maternal characteristics and previous obstetric history. PE was diagnosed as BP >140/90 mmHg and proteinuria >300 mg/dl and IUGR based on in utero growth reduction by US. We used GA-related PlGF cutoff values with a sensitivity of 94,6% and a specificity of 95%, according to the manufacturer.

RESULTS: In normal pregnancies, PlGF increased until 29 to 32 weeks, and then decreased, confirming previous reports. Eleven of the 16 patients "at risk" had an uncomplicated pregnancy; 9 of those had PlGF levels in the normal range and 2 had PlGF below the cutoff. Five patients developed late-PE and only 2 of those, four weeks prior to the clinical onset of symptoms, had PlGF levels below the cutoff. None developed early-PE. PlGF levels in the PE group were significantly lower than those in the control (-95% of average value). In the IUGR group a non homogeneous distribution was found in PlGF levels (-21% of normal average value); Of the 8 women with IUGR, 3 had both PlGF below the cutoff and abnormal bilateral uterine arterial Doppler velocimetry, and 5 had a normal level of PlGF and normal Doppler velocimetry.

CONCLUSIONS: In a clinical setting, our preliminary data show that PlGF is not a good predictor of late-PE. PlGF is associated with IUGR due to impaired

placentation. Further data on PlGF level in the "risk" group is needed to evaluate its role in predicting or diagnosing early-PE. (supported by ASM Associazione Studio Malformazioni Onlus).

T-310

A Prospective Cohort Study Demonstrates That Maternal Plasma Concentrations of Angiogenic/Anti-Angiogenic Factors Have Prognostic Value in Women Presenting with Suspected Preeclampsia to the Obstetrical Triage Area. Tinnakorn Chaiworapongsa,[1,2] Roberto Romero,[1] Nandor G Than,[1] Edgar Hernandez-Andrade,[1,2] Zeynep Alpay Savasan,[1,2] Adi L Tarca,[1] Gaurav Bhatti,[1] Zhong Dong,[1] Sonia S Hassan.[1,2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective The maternal plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), soluble vascular endothelial growth factor receptor-1 and -2 (sVEGFR-1 and -2) have been demonstrated to predict the subsequent development of early onset preeclampsia (PE). In addition, these factors have been proposed to be useful to identify patients presenting to the triage area who will develop severe PE requiring preterm delivery. This study was undertaken to determine if the findings of such a study were valid.

Study design A prospective cohort study included 85 consecutive patients who presented to the obstetrical triage area between 20-36 weeks with the diagnosis of 'rule out PE'. Patients were classified into: 1) those who developed severe PE and required preterm delivery (n=48) and 2) those who remained stable until delivery at term (n=37). Plasma concentrations of PlGF, sEng, sVEGFR-1 and -2 were determined by ELISA. Established reference ranges for analytes and previous proposed cut-off (MoM) were used for analysis.

Results 1) Patients with a plasma concentration of PlGF/sVEGFR-1 ≤ 0.05 MoM were more likely to develop severe PE, requiring preterm delivery after adjusting for confounders [adjusted OR=24 (95% CI 3.7-164.6)]; 2) A plasma concentration of PlGF/sVEGFR-1 < 0.035 MoM had a sensitivity of 89% (16/18) and a specificity of 96% (24/25) to identify patients who delivered < 2 weeks among patients who presented <34 weeks; 3) This cut-off was also associated with a shorter interval-to-delivery (hazard ratio=13); and 4) Among patients who had a plasma concentration of PlGF/sVEGFR-1 ≤ 5% MoM, 6-34% MoM and ≥ 35% MoM for GA, the rates of delivery ≤ 2 weeks were 84% (16/19), 11% (1/9) and 6% (1/15), respectively. The corresponding rates of delivery < 34 weeks were 90% (17/19), 22% (2/9) and 6% (1/15).

Conclusions This study provides compelling evidence that the determination of angiogenic and anti-angiogenic factors have prognostic value in patients presenting to the obstetrical triage area with suspected preeclampsia. These tests can be performed as a point-of-care and change clinical management.

T-311

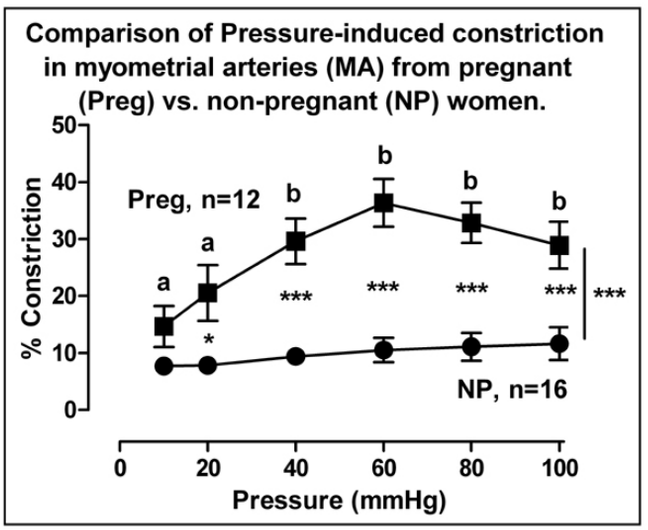
PregnancyIncreases Myometrial Artery (MA) Pressure-Induced Constriction (PIC) Via NOS- or COX-Independent Mechanisms. Delrae M Eckman,[1] Charles R Rosenfeld,[4] Ridhima Gupta,[2] Shelton M Charles,[2] Heather Mertz,[2] Lorna G Moore.[2,3] *[1]Pediatrics, Wake Forest Univ. School of Med.; [2]OB/GYN, Wake Forest Univ. School of Med.; [3]Graduate School of Arts & Sciences, Wake Forest Univ., Winston-Salem, NC; [4]Pediatrics, Univ. of Texas Southwestern Med. School, Dallas, TX.*

Backgound: PIC is a primary modulator of blood flow in the brain, kidney, skeletal muscle, and perhaps other high-flow organs such as the pregnant uterus. PIC is known to be regulated by endothelial-derived factors; namely nitric oxide synthase (NOS)- and/or cyclooxygenase (COX)-products.

Objective: We asked if pregnancy influenced PIC in MA, and if so, whether such an effect could be attributed to alterations in NOS and/or COX by-products.

Methods: MA were isolated from a full thickness biopsy of myometrium collected from 16 nonpregnant (NP) and 12 non-laboring pregnant (P, 39.0±0.2 wks gestation) women undergoing elective hysterectomy for medical indications or cesarean section, respectively. Resistance level (<250 μm) MA were rapidly isolated, cleaned and studied using pressure myography. PIC was assessed by stepwise increases in intralumenal pressure from 10 to 100 mmHg in the absence or presence of nonspecific NOS and/or COX inhibitors, L-NAME (200μM) and indomethacin (10μM), respectively. Data are means ± SEM.

Results: In the absence of NOS and/or COX inhibition, P increased MA PIC compared to that seen in NP women (e.g., % PIC @ 40mmHg: NP 9.4±1.6, n=16 vs. P 29.6±3.9, n=12, p<0.001; see figure). L-NAME had no effect on PIC in MA from NP women, but augmented PIC in MA from P women (% PIC @ 40mmHg: P, 23.4.4.9 vs P+LNAME, 36.1±4.8, n=7, p<0.01). In contrast, indomethacin had no effect on MA from NP or P women when given alone or in combination with L-NAME.



Conclusion: Human pregnancy is associated with increases in PIC in MA that are partially opposed by enhanced NOS activity; thus, neither NOS nor COX signaling is responsible for the augmented PIC observed in MA from P women. Further study of MA reactivity will provide additional insights into its contribution to pathologic conditions that modify maternal placental blood flow and fetal well-being.

T-312

Stromal Cell-Derived Factor-1 (SDF-1) Is Significantly Elevated in Preeclampsia. Julia V Gefter,[2] Carl A Hubel,[1,2] Robert W Powers.[1,2] *[1]Obstetrics, Gynecology, Reproductive Sciences, Magee Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetrics, Gynecology, Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA.*

Context: SDF-1 is a cytokine that activates leukocytes and is often induced by proinflammatory stimuli such as lipopolysaccharide, TNFα, or IL-1. SDF-1 plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow. EPCs have been shown to be lower in women with preeclampsia compared to normal pregnancy, but the mechanism(s) behind this decrease are unclear. We hypothesized that SDF-1 may be dysregulated in preeclampsia, consistent with reported changes in circulating EPCs.

Objective: We investigated the concentration of circulating SDF-1 in mid- and late pregnancy in subjects with and without preeclampsia. We also investigated if there was any association between SDF-1 and soluble c-Kit, another factor that influences EPC mobilization, and that is reported to be significantly lower before and during preeclampsia.

Study Design: Maternal EDTA plasma samples were collected at 18.4±1.9 (mean±SD) weeks and 37.2±3.9 weeks of pregnancy from women who developed preeclampsia (n=20) and from uncomplicated controls (n=39). SDF-1 was measured using a specific ELISA (Abcam). Statistical analysis was by Wilcoxin rank sum, and correlation analysis was performed using Pearson product moment correlation coefficient.

Results: SDF-1 levels were significantly higher in late pregnancy, but not mid-pregnancy, in preeclampsia compared to uncomplicated controls. In contrast, sc-kit levels were significantly lower in mid and late pregnancy. No association was observed between soluble c-Kit and SDF-1.

Conclusion: Dysregulation of SDF-1 and sc-kit in pregnancy may contribute to observed differences in EPCs in preeclampsia.

groups	SDF-1 (pg/ml) mid-pregnancy	SDF-1 (pg/ml) late-pregnancy	sc-kit (pg/ml) mid-pregnancy	sc-kit (pg/ml) late pregnancy
Uncomplicated Controls	219.5±53.1	214.3±44.4	11101±2429	7781±1868
Preeclampsia	229.7±68.1	253.7±73.6*	9503±2582*	5848±1504*

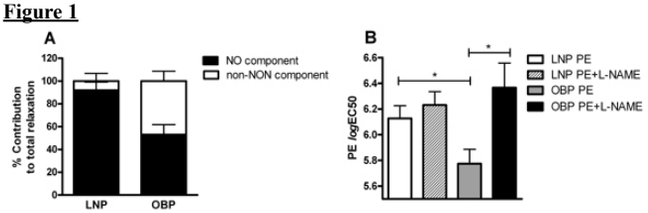
Data are mean±SD. *: p<0.05 compared to controls

This project supported by National Institutes of Health grant R01-HL091094.

T-313

Free Access to Sucrose and Lard Increases Adiposity and Alters Uterine Artery Function in Late Pregnant Rats. Styliani Goulopoulou,[1] Johanna L Hannan,[2] R Clinton Webb.[1] *[1]Department of Physiology, Georgia Health Sciences University, Augusta, GA, USA; [2]The James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, MD, USA.*
Objective: Obesity is associated with endothelial dysfunction and it has been suggested that obese women enter pregnancy with preexisting vascular abnormalities. We hypothesized that feeding a diet rich in lard and sucrose to rat dams before and during pregnancy will increase maternal adiposity and will lead to abnormal uterine artery (UtA) function (reduced relaxation, increased constriction). **Methods:** Female Sprague-Dawley rats were fed for 3 weeks prior to pregnancy and throughout gestation either normal chow (lean pregnant, LNP, n=8) or normal chow plus lard plus liquid sucrose (obese pregnant, OBP, n=7). UtA segments were mounted in a wire myograph and concentration-response curves to acetylcholine (ACh) and phenylephrine (PE) in the presence and absence of nitric oxide synthase (NOS) inhibitor (L-NAME) were performed. **Results:** There were no group differences in litter size (LNP vs. OBP: 14.6 ± 0.62 vs. 13.9 ± 0.67), fetal (3.3 ± 0.54 g vs. 2.9 ± 0.49 g) and placental (0.474 ± 0.021 vs. 0.472 ± 0.022 g) weights. Fat pads weighed more in OBP vs. LNP rats (parametrial: 9.9 ± 0.86 g vs. 5.0 ± 0.32 g; mesenteric: 4.6 ± 0.54 g vs. 2.9 ± 0.18 g; retroperitoneal: 4.8 ± 0.68 g vs. 1.9 ± 0.07 g; omental: 1.34 ± 0.200 g vs. 0.65 ± 0.043 g, $p<0.05$). ACh-induced maximum relaxation was similar between groups (LNP: 92.8 ± 1.92%; OBP: 88.9 ± 3.86%, $p>0.05$). NOS inhibition reduced ACh-induced relaxation in LNP and OBP UtA but this effect was smaller in the OBP rats, suggesting that the NO-component of ACh-induced relaxation is reduced in OBP vs. LNP rats (Fig 1A). UtA sensitivity to PE was lower in OBP vs. LNP rats and NOS inhibition increased sensitivity to PE only in the OBP group (Fig 1B). **Conclusion:** Feeding a diet rich in lard and sucrose to rat dams before pregnancy and throughout gestation increases maternal adiposity, reduces the contribution of the NO-component to UtA relaxation, and alters the role of NO in contractile responses to PE.

Figure 1



*p<0.05, vs. OBP PE.

T-314

Vitamin D Enhances VEGF Expression in Endothelial Progenitor Cells. Magdalena Grundmann,[1] Mariam Haidar,[1] Carl A Hubel,[2] Frauke M von Versen-Hoeynck.[1] *[1]Department of Obstetrics & Gynecology, Hannover Medical School, Hannover, Germany; [2]Magee Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA.*
Background: The main pathogenic feature of preeclampsia seems to be impaired placentation. In addition, preeclampsia is associated with a vitamin D deficiency. Endothelial progenitor cells, in particular their highly proliferative subpopulation of endothelial colony forming cells (ECFC), play an important role in placental vasculogenesis. However, the mechanisms of vitamin D3 influence on placental development are poorly understood. We investigated the influence of vitamin D3 on the differentiation of endothelial progenitor cells (ECFCs) in a placental angiogenesis model and hypothesized that vitamin D3 stimulates the expression of vascular endothelial growth factor (VEGF) in ECFCs.
Methods: Umbilical cord blood was obtained from uncomplicated, term pregnancies, the mononuclear cells were isolated and seeded onto collagen-coated culture plates for outgrowth of ECFCs. After preincubation with 10 nM vitamin D3, ECFCs were plated onto Matrigel (BD Biosciences) in the presence of the treatment media. After 6 hours capillary-like tubules were fixed and their total length was determined per well and median values were calculated from n=38 experiments. For mRNA expression analyses total RNA isolation was performed. High capacity cDNA reverse transcription kit (Invitrogen) was used for cDNA synthesis and Real time RT-PCR was performed on the Rotor Gene 6000 PCR instrument (Corbett Research) using VEGF-A primers according to existing literature. Statistical analysis was performed using Wilcoxon signed rank test.
Results: ECFCs treated with 10nM vitamin D3 showed a 1,27 times higher tubule formation compared to vehicle-treated controls (1.27 ± 0.19, $p< 0.001$, n= 38). mRNA expression analysis showed a 1.9 times higher expression of VEGF-A mRNA in ECFCs treated with 10 nM vitamin D3 compared to controls (1.82 ± 0.43, $p< 0.0001$, n=18).
Conclusion: Physiological concentrations of vitamin D3 significantly promote the formation of capillary-like structures by ECFCs in a cell culture model. This effect is associated with an up-regulation of VEGF-mRNA in ECFCs by vitamin D3. Since the de novo angiogenesis is a crucial step in placental development, vitamin D deficiency could play an important role in the pathophysiology of preeclampsia.

T-315

Changes in Pulse Wave Velocity Resulting from Pregnancy Suggest Pregnancy Induced Vascular Remodeling. Sarah A Hale,[1] Gary J Badger,[2] Carole McBride,[1] Ira M Bernstein.[1] *[1]Ob/Gyn and Reproductive Sci, University of Vermont, Burlington, VT, USA; [2]Medical Biostatistics, University of Vermont, Burlington, VT, USA.*
We have hypothesized that preeclampsia results from poor maternal vascular compliance coupled with the volume expansion of pregnancy and that pregnancy induces vascular remodeling that increases vascular compliance and lowers risk of preeclampsia in future pregnancies. Pulse wave velocity (PWV) is an index of arterial compliance and often used in the general population for assessing risk for cardiovascular disease. Here, we examined PWV prior to pregnancy and 1 year postpartum to determine if it is modified by pregnancy. 43 healthy nulliparous subjects were enrolled in a longitudinal study. Seventeen women subsequently achieved singleton pregnancies, delivered at term and were studied again at 1 yr postpartum, (30 months following prepregnancy evaluation, PREG). Twenty-six women comprised the control group, with follow-up evaluation 30 months after initial testing, with no interval pregnancy (NP). Data are expressed as mean ± SE. $P < 0.05$ was considered statistically significant. We observed no significant differences prior to pregnancy between NP and PREG in maternal age, BMI, mean arterial pressure or PWV. In subjects who had an interval pregnancy PWV was reduced consistent with improved arterial compliance (prepregnancy: 2.73 ± 0.05, postpartum: 2.49 ± 0.05 m/s, $p<0.001$). We observed no difference in PWV over time in NP subjects (first visit: 2.56 ± 0.04, follow up visit: 2.50 ± 0.04 m/s, $p=0.15$). In a separate cohort of 10 prior preterm preeclamptics we evaluated the relationship between PWV and interval from previous pregnancy. Mean interval from delivery to evaluation was 111 ± 20 wks, mean gestational age at delivery was 31.5 ± 1.8 wks and mean maternal age was 31.6 ± 1.6 yrs. Interval was highly correlated with PWV ($r = 0.72$, $p = 0.02$) with longer intervals associated with higher PWV. This is consistent with progressive reversal of pregnancy-induced remodeling. We propose that pregnancy induces vascular remodeling evidenced by increased compliance and decreased PWV postpartum. Vascular remodeling that occurs as a result of a first pregnancy may be protective for a subsequent pregnancy provided that the time interval between pregnancies is not excessively long. This phenomena may explain why risk for preeclampsia decreases after first pregnancy.

T-316

Elevation of Phospholipids in Third Trimester and Correlation with Uterine Blood Flow. Denise G Hemmings,[1] David N Brindley,[2] Jonathan M Curtis,[3] Yeping Xiong,[3] Sarah A Hale,[4] Ira M Bernstein.[4] *[1]Obs/Gyn, Univ of Alberta (UofA); [2]Biochemistry, UofA; [3]Agric Food & Nutritional Science, UofA; [4]Ob/Gyn and Repro Sci, Univ of Vermont.*
Background: Uterine blood flow (UBF) is dramatically elevated during pregnancy and reduced UBF is associated with preeclampsia. Phospholipids including sphingosine 1-phosphate (S1P) and various species of lysophosphatidic acid (LPA) have vasoactive properties, but have not been assessed in relation to UBF. LPA and S1P have dual vascular effects depending on cell surface receptor stimulation on endothelial (vasodilation) vs smooth muscle cells (vasoconstriction). Although our lab and others have proposed the importance of LPA and S1P in pregnancy, few phospholipids have been measured longitudinally in plasma samples from normal or complicated pregnancies.
Hypothesis: Both S1P and LPA species will be elevated in plasma as pregnancy progresses and these levels will correlate positively with UBF in the third trimester (3rd tri).
Methods: Women were recruited prior to or after pregnancy with a subset followed through pregnancy into post-partum: pre-pregnant (23), 1st tri (13), 3rd tri (18), post-partum (41). A small subset of these women (3) developed preeclampsia. UBF was assessed by color Doppler ultrasound longitudinally from pre-pregnancy to post-partum where possible. Lipids were extracted from plasma samples and assessed by mass spectrometry. Average concentrations

were then compared and where available correlated with UBF for: 16:0-LPA, 18:0-LPA, 18:1-LPA, S1P and sphinganine 1-phosphate (SAP).
Results: We found significant increases of 16:0-LPA and 18:1-LPA in 3rd tri compared to all other stages. S1P increased in 3rd tri compared to 1st tri and post-partum. In 3rd tri only, there were significantly positive correlations of UBF with 18:1-LPA, 18:0-LPA and a trend to significance for 16:0-LPA, but not for S1P or SAP. Interestingly, the one preeclamptic sample we measured from 1st tri had nearly double the levels of S1P, SAP and 18:0-LPA compared to samples from normal pregnant women.
Significance: The rise and correlation with increased UBF of lipids with vasodilatory properties in 3rd tri suggests they could play an important role in the normal physiological vascular changes during pregnancy. The finding of dramatically increased levels in 1st tri from a woman with preeclampsia emphasizes the need for further investigation into the role of these lipids in pregnancy.
Supported by NSERC, CIHR and WCHRI

T-317

Vascular Pool of Releasable Soluble VEGF Receptor-1 (sFlt1) Revealed after Intravenous Heparin. Carl A Hubel,[1,3] Robert W Powers,[1,2] Lia R Edmunds,[1] Ashley Myerski,[1] James M Roberts,[1,2] Robin E Gandley,[1,3] Augustine Rajakumar.[4] *[1]Magee-Womens Research Institute, Univ. Pittsburgh; [2]OBGYN-RS, Univ. Pittsburgh; [3]Environmental and Occupational Health, Univ. Pittsburgh, PA; [4]Beth Israel Deaconess Medical Center, , Boston, MA, USA.*
Introduction: The extracellular matrix selectively binds proteins via a heparin-binding domain. We investigated the effect of a heparin bolus on circulating sFlt1 and free VEGF in vivo. **Methods:** Non-pregnant women with prior preeclampsia (n=15) or prior uncomplicated pregnancy (n=16), post first pregnancy, and nulligravidas (n=8) were studied (all non-smokers and non-lactating). After an overnight fast, 70 IU/kg body weight of unfractionated heparin was administered IV, and blood samples obtained both pre- and 15 min. post-heparin. Plasma sFlt1 and free VEGF were measured by ELISA (R&D).
Results: Women with prior preeclampsia had slightly higher systolic blood pressures and pre-heparin (baseline) plasma sFlt1 levels compared to prior uncomplicated pregnancy (Table). sFlt1 was 150-fold elevated post-heparin in all groups, with no difference between groups. Pre- vs. post-heparin sFlt1 differences were confirmed by Western, showing a single 100kd isoform. Increases in sFlt1 were accompanied by decreases in free VEGF in all groups.

Mean (±SD) or median (IQR)	Prior preeclampsia	Prior uncomplicated pregnancy	Nulligravid
Days post delivery	389 ± 114	388 ± 151	
Systolic BP (mm Hg)	*119 ± 10	109 ± 8	109 ± 8
Diastolic BP (mm Hg)	#81 ± 10	73 ± 9	68 ± 9
sFlt-1 (pg/mL) pre-heparin	*42 (37 – 47)	34 (29 - 43)	30 (27 – 40)
sFlt-1 (pg/mL) post-heparin	Ψ 11424 (8299-12833)	Ψ 10003 (8413-12583)	Ψ 9032 (7424-10567)
VEGF (pg/mL) pre-heparin	76 (36 – 191)	51 (42 – 198)	54 (33 – 134)
VEGF (pg/mL) post-heparin	Ψ 15 (9 – 37)	Ψ 18 (10 – 159)	Ψ 19 (7 – 97)

# P<0.05 vs. Nulligravid; * P<0.05 vs. Prior uncomplicated pregnancy; Ψ P<0.01 vs. pre-heparin

Addition of heparin to plasma had no effect on sFlt1 values. Incubation of whole blood with heparin resulted in 10-fold elevations in plasma sFlt1 (P<0.01).
Conclusion: Large reserves of releasable sFlt1 exist in the vasculature, with a minor fraction from blood components. Heparin resulted in sFlt1 levels in non-pregnant women similar to those observed during late-pregnancy, accompanied by a decline in free VEGF. This sFlt1 pool, likely electrostatically bound to heparan sulfate proteoglycans of the endothelial glycocalyx, may impact therapeutic strategies to prevent or reverse pathologies such as preeclampsia.

T-318

Cell-Free Hemoglobin Is Elevated in HELLP Syndrome but Not Preeclampsia. Arun Jeyabalan,[1] Robert W Powers,[1] Robin E Gandley,[1] Carl A Hubel,[1] Mark T Gladwin.[3] *[1]Magee-Womens Research Institute, Dept of OBGYN-RS, University of Pittsburgh; [2]Vascular Medicine Institute and Dept of Medicine, University of Pittsburgh.*
Background: Normal pregnancy is a state of systemic vasodilation mediated in part by nitric oxide (NO). Reduced NO bioavailability may be responsible for abnormal vascular adaptations to pregnancy and pathologic pregnancy states such as preeclampsia. Cell-free hemoglobin rapidly reacts with NO leading to reduced NO bioavailability and subsequent endothelial dysfunction and vasoconstriction, a proposed mechanism in the pathophysiology of several vascular and hemolytic diseases.
Objective: The aim of this study was to determine whether cell-free hemoglobin is elevated in preeclampsia or HELLP syndrome compared to uncomplicated pregnancies.
Study Design: We conducted a case-control study of 10 women with HELLP syndrome, 9 women with severe preeclampsia, and 9 with mild preeclampsia, with each case matched to a normal pregnancy (control) subject based on gestational age at sample collection, race and parity. Serum cell-free hemoglobin was measured by enzyme-linked immunosorbent assay. Data are presented as median [interquartile range] and analyzed by Wilcoxon sign rank test for matched pairs.
Results: Baseline demographic characteristics were similar in all groups except for the earlier gestational age at delivery in women with severe preeclampsia and HELLP syndrome. Cell-free hemoglobin was 50% higher in all preeclampsia and HELLP syndrome combined compared to controls (1.99μM [1.13-5.45] vs. 1.54μM [0.95-1.92], p=0.01). Women with HELLP syndrome had 3.6-fold higher cell-free hemoglobin compared to matched controls (p=0.04). There was no significant difference in cell-free hemoglobin in mild and severe preeclamptic women compared to their respective controls.

Cell-free hemoglobin (μM)

Category	Cases	Controls	p-value
HELLP syndrome	6.36 [1.04, 12.71]	1.75 [1.00, 2.07]	0.04
Severe preeclampsia	1.17 [1.07, 2.54]	1.27 [0.82, 1.58]	0.14
Mild preeclampsia	1.99 [1.49, 2.41]	1.58 [1.45, 1.69]	0.51

Conclusions: Circulating cell-free hemoglobin is elevated in HELLP syndrome compared to uncomplicated pregnancies, but not in preeclampsia. This is likely secondary to hemolysis associated with HELLP syndrome. We speculate that this contributes to reduced NO bioavailability, vasoconstriction, oxidative stress, and the progression of maternal disease including organ dysfunction associated with HELLP syndrome.

T-319

Infused Sphingosine 1-Phosphate Increases Endothelial Permeability in Pressurized Arteries Isolated from Non-Pregnant and Pregnant Mice Infected with Cytomegalovirus. Daniel Kerage,[1,3] Randi Gombos,[2,3] Denise G Hemmings.[2,3] *[1]Med Micro and Immun, University of Alberta; [2]Physiology, UofA; [3]Obs/Gyn, UofA.*
Introduction: Cytomegalovirus (CMV) infection increases vascular endothelial permeability, which is implicated in the pathogenesis of diseases including cancer, atherosclerosis and preeclampsia. Vascular endothelial growth factor (VEGF), a known permeability factor, is increased in these diseases and also during CMV infection. However, it is not known if the increase in CMV-induced VEGF is associated with increased endothelial permeability. Both CMV and VEGF can also activate sphingosine kinase-1 producing sphingosine 1-phosphate (S1P), a bioactive lipid that regulates endothelial permeability. However, direct evidence that CMV activates S1P alone or through VEGF to increase endothelial permeability is lacking. We hypothesized that endothelial permeability in intact isolated arteries from CMV-infected mice will be increased in response to VEGF and S1P compared to those from uninfected mice and that this increase will be enhanced in arteries from late pregnant mice.
Method: We studied endothelial permeability in arteries isolated from uninfected and CMV-infected nonpregnant (NP) or late pregnant (LP) mice using Evan's Blue Dye (EBD) infused into pressurized arteries mounted on a pressure myograph. VEGF or S1P were infused into arteries in the presence of EBD and leakage of the dye into vascular wall was measured by optical density.
Results: VEGF (0.1 μM) increased permeability in uterine and mesenteric arteries from uninfected NP mice compared to EBD control, but not in arteries from infected NP mice. VEGF-induced permeability was increased in uterine arteries from infected compared to uninfected LP mice. Infusion of 0.1 or 1μM S1P into uterine arteries from uninfected NP and LP mice, and mesenteric arteries from uninfected LP, but not NP, showed increased endothelial permeability compared to 0.01μM S1P but not to the EBD control. Infusion of 0.1μM S1P into uterine and mesenteric arteries from infected NP and uterine arteries from LP mice also showed increased permeability.
Conclusion: The increased VEGF and S1P-induced permeability in uterine arteries from infected LP mice indicates that CMV could be increasing both VEGF and S1P receptor expression. This suggests that infections could contribute to endothelial dysfunction and pregnancy disorders such as intrauterine growth restriction and preeclampsia.
Supported by CIHR and WCHRI

T-320

VEGF Stimulates Acute Nitric Oxide (NO) Production and Inhibits Subsequent ATP Stimulated NO Production in Human Umbilical Vein Endothelium (UV Endo) to a Level Observed in Subjects with Preeclampsia. Jennifer Krupp, Derek Boeldt, FuXian Yi, Dinesh Shah, Ian Bird. *Dept of OB/GYN, University of Wisconsin, Madison.*

Objective: Pregnancy adaptation of the vasculature allows increased blood flow to the uterus to support the placenta and fetus. Uterine artery (UA) endothelial cells from pregnant sheep show sustained Ca2+ bursting in response to ATP stimulation leading to increased production of the vasodilator NO. We have shown in sheep UA endothelium, VEGF promotes a Ca2+ response and subsequent responses to ATP are blunted by VEGF pretreatment. When intact Human UV Endo from normal term subjects is treated with ATP, a similar response is seen showing sustained Ca2+ bursts and an associated increase in NO production over 30 minutes. Ca2+ and NO responses are lacking in UV Endo from Human subjects with preeclampsia (PE). Given VEGF is elevated in PE subjects, the goal of this study is to establish if VEGF can induce PE type dysfunction in otherwise normal UV Endo.

Results: Cultured human umbilical vein endothelial cells (HUVEC) from term normal pregnancy show similar Ca2+ responses when treated with ATP as are seen in UV Endo. In pooled HUVEC, over 90% of the cells respond to VEGF (10ng/ml) with increased Ca2+ production or sustained Ca2+ bursting for 30 minutes. With U73122 (Phospholipase C inhibitor) pretreatment, the response to VEGF was blunted but not fully blocked. In UV Endo, VEGF stimulated a similar Ca2+ rise accompanied by a rise in NO production the magnitude of which is proportionate to the rise in Ca2+. However, when UV Endo was stimulated with ATP after treatment with VEGF, the subsequent response to ATP was inhibited at the level of both Ca2+ and NO, becoming similar to what we previously reported in PE subjects.

Conclusion: Regarding VEGF signaling mechanisms, these findings suggest the Ca2+ signaling in HUVEC in response to VEGF is to some extent IP3 dependent. Continued Ca2+ elevation and bursting in response to VEGF after U73122 treatment suggests the Ca2+ response is not just mediated by IP3. Physiologically, VEGF is clearly also an acute stimulator of NO production in intact UV Endo, suggesting it is not just a stimulator of angiogenesis but also of vasodilation. Nonetheless, of clinical relevance, exposure to VEGF can also inhibit subsequent Ca2+ and NO responses to ATP resulting in the loss of function to other agonists that may far outweigh the direct action of VEGF alone. Funded by NIH R21 HD069181-01

T-321

An Anti-Angiogenic State Is Present in Late-Onset Fetal Death Weeks before the Diagnosis. Jennifer Lam,[1,2] Tinnakorn Chaiworapongsa,[1,2] Nandor G Than,[1] Lami Yeo,[1,2] Juan Pedro Kusanovic,[1] Adi L Tarca,[1] Gaurav Bhatti,[1] Sonia S Hassan,[1,2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective Stillbirth accounts for half of perinatal deaths; 1/3 of cases occur near term. Common risk factors are growth restriction and placental abruption. Changes in angiogenic and anti-angiogenic factors have been reported in pregnancies complicated with lesions of placenta underperfusion (i.e. preeclampsia, intrauterine growth restriction and placental abruption). The aim of this study was to determine whether changes in plasma concentrations of angiogenic and anti-angiogenic factors occur prior to fetal death.

Study design A case-control study was designed in patients who have been enrolled in a longitudinal study. Cases were patients who had a fetal death after 34 weeks (n=5). Controls consisted of women with uncomplicated pregnancies who delivered at term (n=30). Blood samples had been obtained between 30-34 weeks. Cases were matched to controls (1:6) by gestational age (GA) at sampling (≤ 2 weeks), race, parity, tobacco use and BMI. Plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), and soluble vascular endothelial growth factor receptor (sVEGFR)-1 were measured by ELISA.

Results Patients who subsequently had a fetal death had a lower median plasma concentration of PlGF compared to controls (p=0.016). In contrast, the median plasma concentration of sVEGFR-1 and sEng was significantly higher in cases with a subsequent fetal death than in the control group (p=0.016 and 0.002). A PlGF/sVEGFR-1 ratio of ≤0.047 or PlGF/sEng ratio of ≤ 11.7 had a sensitivity of 80% and a specificity of 93% to identify stillbirth in the third trimester.

Descriptive Statistics

	Controls	Fetal Death
GA at blood sampling	32.9 (32.1-33.6)	33.4 (32-33.7)
PlGF (pg/mL)	646 (279-1108)	97 (63-640)*
sVEGFR-1 (pg/mL)	2779 (1822-4349)	6333 (3740-6908)*
sEng (ng/mL)	7.5 (5.7-10.1)	23.8 (14.4-33.4)*
PlGF/sVEGFR-1 ratio	0.25 (0.09-0.5)	0.02 (0.009-0.1)*
PlGF/sEng ratio	96 (28-167)	6.9 (2.3-28)*

Median (interquartile); *p<0.05

Conclusion An anti-angiogenic state is present prior to fetal death in the third trimester of pregnancy. These biomarkers allow the identification of fetuses at risk and testing of intervention to prevent stillbirth.

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Fetal Loss of Adrenomedullin Causes Maternal Vascular Pathology Typical of Preeclampsia. Patricia Lenhart, Manyu Li, Nicole Schwerbrock, Kimberly Fritz-Six, Mahita Kadmiel, Kathleen Caron. *Cell and Molecular Physiology, University of North Carolina Chapel Hill.*

Adrenomedullin (AM) is a 52-amino acid peptide vasodilator that is elevated 3 to 5-fold in normal human pregnancies but is often blunted in pregnancy complications such as fetal growth restriction, gestational diabetes, and preeclampsia. Our previous studies have shown that reduced maternal AM leads to poor pregnancy outcomes, but the role of fetal AM remains unknown. Therefore, we examined placentas of $Adm^{-/-}$ and $Adm^{+/+}$ pups at midgestation for structural, functional, and developmental phenotypes. Histologic analysis revealed normal differentiation and layer formation in the $Adm^{-/-}$ placentas. Doppler ultrasound of umbilical cord blood flow showed that placental impedance was unaffected by fetal loss of *Adm*. Histology and lectin staining revealed that the fetal vessels of the $Adm^{-/-}$ placentas were large and underbranched compared to those of $Adm^{+/+}$ littermates. Electron microscopy of methyl-methacrylate casts confirmed that $Adm^{-/-}$ placentas had reduced fetal vessel branching; a hallmark feature of preeclampsia. Strikingly, the $Adm^{-/-}$ placentas also had significantly reduced decidual natural killer (NK) cells and reduced remodeling of maternal spiral arteries; other known features of preeclampsia. To further study the effects of AM on NK cells, isolated primary NK cells were treated with AM, which resulted in significantly altered cytokine expression. Primary smooth muscle cell cultures exposed to supernatant from AM-treated NK cells showed a significant increase in apoptosis, indicating that the AM-mediated change in NK cell cytokine expression may be required for spiral artery remodeling. To confirm that this phenotype is due to lack of fetal AM, ovarian transplantation was performed from $Adm^{+/-}$ to $Adm^{+/+}$ females. The placentas of $Adm^{-/-}$ pups from $Adm^{+/+}$ mothers receiving $Adm^{+/-}$ ovary transplants recapitulated the reduced NK cell invasion and failed spiral artery remodeling seen in $Adm^{+/-}$ mothers, demonstrating that lack of fetal AM is responsible for the observed pathology. We reversed this phenotype by generating a mouse model with increased fetal AM expression, termed $Adm^{hi/hi}$. High fetal AM led to increased decidual NK cells and normal spiral artery remodeling. Taken together, these findings demonstrate that the dosage of fetal AM is a critical factor for adapting the maternal vasculature to pregnancy and may be important in preventing the development of preeclampsia.

T-323

Progression of the Plasma Angiogenic/Anti-Angiogenic Factor Concentrations after the Diagnosis of Preeclampsia: A Longitudinal Study. Yi Li,[1,2] Tinnakorn Chaiworapongsa,[1,2] Zeynep Alpay Savasan,[1,2] Adi L Tarca,[1] Zhong Dong,[1] Sonia S Hassan,[1,2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective An imbalance of angiogenic/anti-angiogenic factors in maternal serum/plasma is observed both prior to and at the time of diagnosis of preeclampsia (PE). However, the changes of these factors after the diagnosis have never been reported. This study examines the changes of placental growth factor (PlGF), soluble endoglin (sEng), soluble vascular endothelial growth factor receptors (sVEGFR)-1 and -2 longitudinally after the diagnosis of PE.

Study design The study included 88 patients in the following groups: 1): mild PE who remained stable until delivery at term (n=29; 99 samples); 2): mild PE who developed severe disease prior to delivery. This group was subdivided into two groups according to the interval-to-delivery: 2A (delivery within two weeks: n=16, 35 samples and 2B (delivery after two weeks: n=10; 35 samples); 3): severe preeclampsia (n=33; 87 samples). Plasma concentrations of PlGF, sEng, sVEGFR-1 and -2 were determined by ELISA and adjusted for gestational age (using a reference range constructed from our population, n=180; 1,046 samples). Mixed effect model was used for data analysis.

Results 1) At presentation, the mean multiple of median (MoM) plasma concentrations of PlGF and PlGF/sVEGFR-1 ratio were significantly lower in Group 2B and Group 3 than in Group 1 ($p<0.05$). The mean MoM plasma concentrations of sVEGFR-1 and sEng were significantly higher in Group 2B than in Group 1 ($p<0.001$); 2) all groups had similar changes over time (slope) in MoM plasma PlGF concentration ($p>0.05$); and 3) in contrast, Group 2B (mild PE who delivered ≤ 2 weeks) and Group 3 (severe PE) had significantly higher mean slopes of MoM plasma sVEGFR-1 concentration than in Group 1 (mild PE who delivered at term) ($p<0.001$).
Conclusions Plasma concentrations of angiogenic/anti-angiogenic factors at diagnosis of PE may be useful to determine the prognosis in terms of progression to severe disease and delivery within 2 weeks. The evolution of PlGF concentration over time did not differ between those with mild PE who remained stable and those with mild PE who developed severe disease. In contrast, the anti-angiogenic factor, sVEGFR-1 concentration, increased significantly over time in those who developed severe disease and delivered within 2 weeks after diagnosis.

T-324

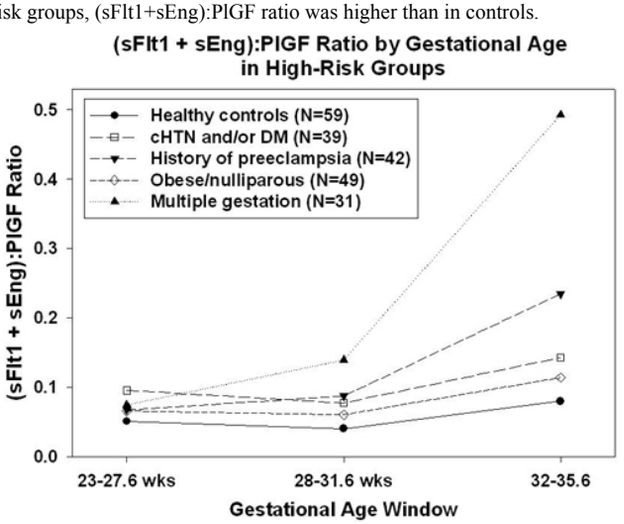
Roles of Aryl Hydrocarbon Receptor in Human Fetal Endothelial Cell Functions. Yan Li, Kai Wang, Yingjie Zhao, Huihui Li, Dongbao Chen, Jing Zheng. *Dept. of Ob/GYN, Univ. of Wisconsin, Madison, WI, USA.*
Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, is a classic receptor mediating dioxin (TCDD)-induced adverse effect on pregnancy, causing increases in fetal and neonatal mortality and decreases in little sizes. However, AhR knockout in mice also leads to similar adverse phenotypes in the fetus and newborn, possibly partially due to abnormal vascular development. Herein, to examine physiological roles of AhR in fetal vasculature, we test the **hypothesis** that endogenous AhR ligands 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) and TCDD suppress endothelial cell growth, migration, and tube-formation and affect expression of eNOS in human umbilical cord vein (HUVE) and artery (HUAE) cells. **Methods:** The expressions of AhR in HUVE & HUAE cells as well as normal human term placentas were evaluated by immunohistochemistry and Western blotting. Cell proliferation and migration were determined using the crystal violet cell proliferation assay and the BD FluoroBlok Trans-wells system respectively. eNOS protein expression was evaluated by Western blotting. **Results:** We found that the AhR was immunolocalized in trophoblast cells and vascular endothelial cells of human placentas and umbilical cord Vessels. Both ITE and TCDD dose- and/or time-dependently inhibited ($p \leq 0.05$) HUAEC and HUVEC cell proliferation, while both ITE and TCDD inhibited ($p \leq 0.05$) HUAEC, but not HUVEC cell migration. A single dose of ITE and TCDD decreased ($p \leq 0.05$) AhR protein levels in HUVEC and HUAEC cells, indicating activation of the AhR. ITE increased eNOS protein levels ($p \leq 0.05$) in HUVEC while decreased eNOS protein levels in HUAEC ($p \leq 0.05$). **Conclusions:** These data indicate that ITE and TCDD suppress HUVE and HUAE cell proliferation, whereas differentially regulate HUVE and HUAE migration. ITE also differentially regulate eNOS protein expression in HUAE and HUVE cells. Thus, AhR may play important and differential roles in regulating fetal artery and vein endothelial cell functions. Supported by NIH HD38843 (JZ).

T-325

LRRC26 Increases BK_{Ca} Channel Activity in Mouse Uterine Artery Leading to Vasodilation during Pregnancy. Ramon A Lorca,[1] Daniel W Nuno,[2] Kathryn G Lamping,[2] Sarah K England.[1] *[1]Department of Obstetrics and Gynecology, Washington University in St. Louis School of Medicine, St. Louis, MO, USA; [2]Department of Veterans Affairs Iowa City Health Care System, Roy J and Lucille A Carver College of Medicine, University of Iowa, Iowa City, IA, USA.*
The uterine artery (UA) undergoes marked vasodilation during pregnancy to provide an appropriate supply of maternal blood to the fetus, however, the underlying mechanism for the vascular remodeling remains unclear. The large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel is highly expressed in most vascular smooth muscle cells (VSMCs) where it participates in the maintenance of myogenic vascular tone to buffer depolarization and vasoconstriction caused by activation of voltage-dependent Ca^{2+} channels. BK_{Ca} channel expression is increased in the UA during pregnancy and its inhibition reduces basal uterine blood flow. Despite the presence of this channel in UA, its role and regulation during pregnancy and how it contributes to vasodilation is unknown. The leucine-rich repeat-containing (LRRC) protein 26 is a novel modulatory subunit of BK_{Ca} channels. Recently LRRC26 was described to enhance BK_{Ca} activation in a prostate cancer cell line, where it activates BK_{Ca} channels at resting membrane potentials. We have found that mouse LRRC26 (mLRRC26) is present in uterine artery and its expression increases during pregnancy. We hypothesize that mLRRC26 enhances BK_{Ca} channel activity in VSMCs of UA from pregnant mice and may underlie the increased vasodilation seen during pregnancy. We observed that, similar to human LRRC26, mLRRC26 enhances BK_{Ca} channel activation in co-transfected HEK293T cells, producing a leftward shift of the voltage-activation curve of ~100mV. In freshly dissociated UA VSMCs from pregnant mice, iberiotoxin (IbTX), a selective blocker of BK_{Ca} channels, induces a 1.6-fold greater blockade of voltage-dependent currents than in non-pregnant VSMCs. IbTX induces a greater reduction of UA diameter in pregnant compared to non-pregnant mice, implying an increased activity of BK_{Ca} in UA during pregnancy. These results provide new insights into the mechanisms involved in the pregnancy-dependent vasodilation of UA and may provide information about novel modulators that regulate UA vasodilation.

T-326

Mid-Gestation Angiogenic Biomarker Levels Are Increased in Women at High Risk for Preeclampsia. Sharon Maynard,[2] Sybil Crawford,[3] Susie Bathgate,[4] Jing Yang,[5] Laura Robidoux,[1] Melissa Moore,[5] Tiffany Moore Simas.[1] *[1]Obstetrics and Gynecology, University of Massachusetts Medical School; [2]Medicine, Lehigh Valley Health Network; [3]Behavioral and Preventative Medicine, University of Massachusetts Medical School; [4]Obstetrics and Gynecology, George Washington University School of Medicine; [5]Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School.*
Significance: Several maternal risk factors are associated with preeclampsia (PE), but the contribution of placental angiogenic factors to risk in these settings is unknown.
Objective: To compare maternal serum angiogenic biomarker levels in women with major risk factors for PE and healthy controls.
Methods: Women presenting for prenatal care were enrolled if they had one of the following PE risk factors: chronic hypertension (cHTN), nulliparity with pre-pregnancy BMI>30 (obesity/nulliparity), pregestational diabetes mellitus (DM), multiple gestations (MG), or prior PE. Healthy control pregnancies were enrolled for comparison. Serum samples were collected at 3 pre-specified gestational windows between 23 and 36 weeks. sFlt1, sEng, and PlGF were measured by ELISA. The (sFlt1+sEng):PlGF ratio was calculated and compared for each risk group at each gestational window.
Results: Analysis included 22 with cHTN, 49 with obesity/nulliparity, 12 with DM, 31 with MG, 42 with prior PE, and 59 health controls. For all five high risk groups, (sFlt1+sEng):PlGF ratio was higher than in controls.



Biomarker ratio levels were highest in subjects with MG and prior PE, and became more pronounced as gestation progressed. Women with DM, cHTN, and obesity/nulliparity had more subtle differences as compared with controls.
Conclusion: Women with preeclampsia risk factors had higher angiogenic ratios compared with healthy control women. Women with prior preeclampsia and multiple gestations had more extreme elevations of the angiogenic ratio compared with other risk groups consistent with a placental source of disease in these cases. This study illuminates the interplay between risk factors and placental angiogenic biomarkers in the pathogenesis of preeclampsia.

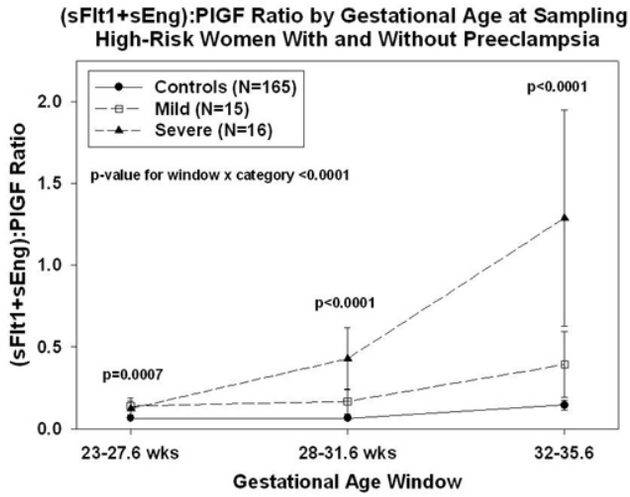
T-327

Angiogenic Biomarkers Are Altered Prior to Preeclampsia Onset in High Risk Women. Tiffany Moore Simas,[1] Sybil Crawford,[2] Susanne Bathgate,[3] Jing Yang,[4] Laura Robidoux,[1] Melissa Moore,[4] Sharon Maynard.[5] *[1]Obstetrics and Gynecology, University of Massachusetts Medical School; [2]Behavioral and Preventative Medicine, University of Massachusetts Medical School; [3]Obstetrics and Gynecology, George Washington University School of Medicine; [4]Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School.*

Significance: Angiogenic biomarkers are increased prior to preeclampsia onset in low-risk women, but there are few studies of high-risk women. Our objective was to determine if maternal serum angiogenic biomarker levels were predictive of preeclampsia in women with major preeclampsia risk factors.

Methods: Women presenting for prenatal care were enrolled if they had one of the following risk factors: chronic hypertension, nulliparity with pre-pregnancy BMI>30, diabetes mellitus, multiple gestations, or prior preeclampsia. Serum samples were collected at three pre-specified gestational windows between 23 and 36 weeks gestation. Maternal serum sFlt1, sEng, and PlGF were measured by ELISA. The development of mild and severe preeclampsia was determined by chart review using standard pre-specified criteria. Geometric mean biomarker levels, and the (sFlt1+sEng):PlGF ratio were compared among women who developed mild, severe, and no preeclamspia

Results: Complete outcome and biomarker data were available for 195 women; of these, 15 developed mild and 16 developed severe preeclampsia. Women who developed preeclampsia had significantly higher serum levels of sEng and sFlt1, and lower levels of PlGF, as compared to women who did not develop preeclampsia, at all gestational ages examined. The sFlt1+sEng:PlGF ratio was consistently highest in women who developed severe preeclampsia; differences were present at 23-27.6 weeks, and became more pronounced as pregnancy progressed.



Conclusions: Angiogenic biomarker levels are altered prior to preeclampsia onset in high risk women, and the degree corresponded with preeclampsia severity. This study suggests biomarkers may be predictive of preeclampsia in high-risk women, who could benefit substantially from an effective screening test for preeclampsia.

T-328

Gestational Vasoconstriction of the Brachial Artery at Low-Flow; a Novel Observation That Suggests Shear Stress Has a Primary Role in the Vasodilatation of Healthy Pregnancy. Muna Noori,[1] Ann E Donald,[2] Aroon D Hingorani,[3] David J Williams.[4] *[1]Centre for Fetal Care, Queen Charlotte's and Chelsea Hospital, Imperial College NHS Trust, London, United Kingdom; [2]Department of Clinical Pharmacology, St Thomas' Hospital, London, United Kingdom; [3]Department of Epidemiology and Public Health, University College, London, United Kingdom; [4]Institute for Women's Health, University College London Hospital, London, United Kingdom.*

Introduction During healthy pregnancy there is marked and progressive vasodilatation leading to increased blood flow to maternal organs, in particular the utero-placental circulation. While using a measure of flow-mediated dilatation (FMD) to assess endothelial function in pregnancy, we noticed vasoconstriction of the brachial artery during low-flow, induced by **distal** cuff occlusion. Technological improvements in measuring and analysing vascular responses to shear stress allowed us to quantify these novel changes that are so far, unique to pregnancy.

Methodology Endothelial function was prospectively assessed using FMD in 40 healthy, non-smoking pregnant women from 10 weeks gestation, at 4-6 weekly intervals throughout pregnancy and at 15 weeks postpartum. The distal brachial artery was occluded for 5 minutes and brachial artery diameter was measured continuously for 1 minute prior to occlusion, during vessel occlusion and for 5 minutes after cuff release. Maternal heart rate was measured during this time, and blood flow and reactive hyperaemia responses were calculated.

Results A novel and unexpected observation was vasoconstriction of the brachial artery at low-flow, proximal to cuff occlusion. As peripheral blood flow increased, this vasoconstriction became more marked with advancing gestation (ANOVA $p<0.001$) and fell postpartum. As previously observed, maternal heart rate increased and reactive hyperaemia decreased as pregnancy advanced (ANOVA $p<0.001$ in both cases). FMD was unchanged during pregnancy but fell postpartum.

Conclusions Reducing peripheral blood flow in pregnancy leads to proximal artery vasoconstriction. This observation supports the view that high flow shear stress mediates at least part of the progressive vasodilatation of healthy pregnancy.

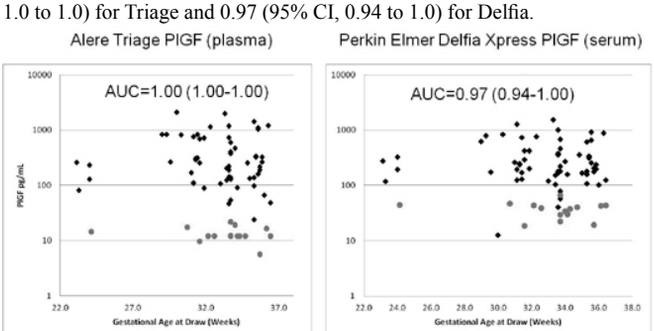
T-329

Comparison of Two Placental Growth Factor Immunoassays as an Aid in the Diagnosis of Preterm Preeclampsia. Jenny E Myers,[1] Louise C Kenny,[2] Lesley ME McCowan,[3] Eliza HY Chan,[3] Gus A Dekker,[5] Lucilla Poston,[6] Nigel AB Simpson,[7] Robyn A North.[6] *[1]Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom; [2]The Anu Reseach Centre, University College Cork, Cork, Ireland; [3]Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand; [4]Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, Australia; [5]Women's Health Academic Centre, King's College London, London, United Kingdom; [6]Department of Obstetrics and Gynaecology, University of Leeds, Leeds, United Kingdom.*

Objectives To compare the diagnostic performance of two commercially available placental growth factor (PlGF) immunoassays.

Materials and Methods A case cohort study was performed in a prospective multicentre cohort of 3572 low risk nulliparous women with a singleton pregnancy (SCOPE study). In fifteen women meeting the **main outcome measure of** preterm preeclampsia, blood samples were collected at the time of diagnosis (ToD). Controls (N=58) were selected from women without preterm preeclampsia and matched for gestational age to cases at a ratio of 4:1. Placental growth factor (PlGF) was measured on Triage® PlGF (Alere) and Delfia® Express PlGF (Perkin Elmer) immunoassay in plasma (Triage) and serum (Delfia). Biomarker levels in cases and controls were compared using median, interquartile range, and Receiver Operator Characteristic (ROC) curves.

Results Median PlGF concentrations in cases and controls were [Triage; 12 (IQR 12-15) v 256 (IQR 130-781) pg/ml, and Delfia; 38 (IQR 28-43) v 236 (IQR 161-419) pg/ml], respectively. PlGF levels were not different between PE with and without SGA measured by either assay. The area under the ROC curve (AUC) for the diagnosis of early onset preeclampsia was 1.0 (95% CI, 1.0 to 1.0) for Triage and 0.97 (95% CI, 0.94 to 1.0) for Delfia.



Conclusions The Triage PlGF assay achieves greater separation of cases from controls. Further studies are needed to understand whether differences in analytical specificity (PlGF isoforms or complexed vs non-complexed PlGF) explain the lower levels of PlGF in preeclampsia for the Triage immunoassay.

T-330

Prediction of the Preterm Preeclampsia in a Prospective Cohort of Nulliparous Women. Robyn A North,[1] Louise C Kenny,[2] Lesley ME McCowan,[3] Eliza HY Chan,[3] Gus A Dekker,[5] Lusilla Poston,[1] Nigel AB Simpson,[6] Jenny E Myers.[7] *[1]Women's Health Academic Centre, King's College London, United Kingdom; [2]The Anu Research Centre, University College Cork, Ireland; [3]Obstetrics and Gynaecology, University of Auckland, New Zealand; [4]Obstetrics and Gynaecology, University of Adelaide, Australia; [5]Obstetrics and Gynaecology, University of Leeds, United Kingdom; [6]Maternal & Fetal Health Research Centre, University of Manchester, United Kingdom.*

Angiogenic factors have been reported to be predictive of preterm preeclampsia (PE), but data are inconsistent and often uncomplicated pregnancies are the comparison group resulting in overestimation of predictive performance.

Objectives To determine the potential of placental growth factor (PlGF), sEndoglin and VEGFR-1, in combination with clinical risk factors and ultrasound data, to predict preterm PE.

Methods Low risk nulliparous women (n=3572) with a singleton pregnancy were recruited into the SCOPE study. A case cohort design was used; cases were defined as women with PE delivered <37 weeks and controls were randomly selected at a ratio of 4:1 from women without preterm PE. Clinical risk factor data were obtained at 14-16 and ultrasound performed at 19-21 weeks. PLGF (Triage®), sEndoglin and VEGFR-1 were measured by immunoassay (Alere) in plasma collected at 14-16 & 19-21 weeks. Prediction models were developed using stepwise logistic regression.

Results Pregnancy outcome was known in 3529 (99%). 47 (1.3%) developed preterm PE. Controls (n=188) comprised uncomplicated and complicated pregnancies, including term PE (4%), SGA (9%), spontaneous preterm birth (4%) and gestational diabetes (2%). Median (IQR) PlGF and sEndoglin (14-16w) in cases and controls were 25 (17-48) v 55 (33-90) pg/ml, p<0.0001 & 71 (56-83) v 62 (54-72) ng/ml, p=0.005 respectively. AUROC values are shown in the table.

	AUC (95%CI) Biomarkers	AUC Clinical Risk + Biomarkers
Clinical risk factors	-	0.83 (0.76-0.89)
PlGF (14-16w)	0.76 (0.68-0.83)	0.90 (0.84-0.94)
sEndoglin (14-16w)	0.63 (0.53-0.72)	0.84 (0.77-0.90)
VEGFR-1 (14-16w)	0.57 (0.47-0.66)	0.83 (0.76-0.89)
PlGF (14-16w) + sEndoglin (19-21w)	0.79 (0.72-0.86)	0.91 (0.87-0.96)
PlGF + sEndoglin + uterine Doppler	-	0.92 (0.88-0.96)

Conclusions The combination of PlGF and clinical risk factors shows promise as a predictor of preterm PE in low risk nulliparous women; additional biomarkers will be required to improve positive predictive values.

T-331

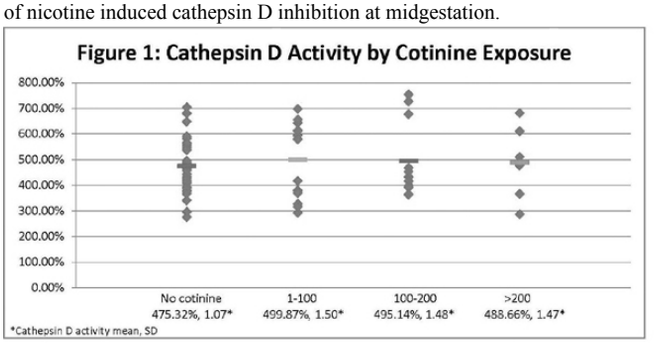
Level of Nicotine Exposure Does Not Alter Human Serum Cathepsin-D Activity in Mid-Gestation. Christy Pearce,[1] Kristine Lain,[2] Linah Al-Alem,[1] Rebecca Epstein,[1] Samantha Mast,[1] John O'Brien,[1] Karen Playforth,[1] Wendy Hansen,[1] Thomas Curry.[1] *[1]OB/GYN, University of Kentucky, Lexington, KY, USA; [2]MFM, Norton Healthcare, Louisville, KY, USA.*

Background: Activated cathepsin D is an enzyme important for the formation of anti-angiogenic prolactin fragments in human serum. Anti-angiogenic prolactin has been implicated in the pathogenesis of severe preeclampsia and its resulting end organ damage. Literature suggests that cathepsin D activity may be inhibited by nicotine. Preeclampsia risk has been reported to be decreased in a dose-dependent fashion in those pregnant women who smoke. We chose to examine the relationship of nicotine exposure (via cotinine levels) and cathepsin D activity in pregnancy at midgestation.

Methods: Women between 16-22 weeks gestation were recruited who self-reported to be smokers (n=29) and non-smokers (n=31). Serum was obtained for measurement of cotinine and cathepsin D activity. Cotinine was reported in ng/ml after measurement with Immulite 2000. Cathepsin-D activity was reported as percentage of control well after fluorescence measurement with SensoLyte® 520 Cathepsin D Assay Kit. Statistical analysis included Student's t-test, ANOVA, Tukey-Kramer, and linear regression.

Results: There was no difference in the mean cathepsin D activity level between those women with cotinine exposure (495.89% ± 1.44) and those without (475.33% ± 1.07), p=0.53, even when stratifying by level of cotinine exposure (see figure 1), p>0.9 for all groups. Linear regression of cotinine and cathepsin D activity as continuous variables showed no trend, r-squared = 0.0059.

Discussion: In this sample at midgestation, smoking exposure does not decrease serum cathepsin D activity. Though cathepsin D may still be implicated in the pathophysiology and even pathogenesis of preeclampsia, the attenuated risk of preeclampsia seen in smokers does not appear to be mediated by a pathway of nicotine induced cathepsin D inhibition at midgestation.



T-332

Endothelial-Dependent Vascular Function Is Significantly Impaired in Obesity and Restored by Overexpression of DDAH1: Evidence for the Role of ADMA. Robert W Powers, Julia Gefter. *Magee-Womens Research Institute, Dept OBGYN-RS, University of Pittsburgh, Pittsburgh, PA, USA.*

Context: Obesity is a significant risk factor for preeclampsia. Vascular dysfunction is a central pathophysiological feature of preeclampsia, and common in obesity. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase, metabolized by the enzyme DDAH1, and significantly elevated in obesity and preeclampsia.

Objective: The focus of this study was to investigate the role of ADMA in obesity-mediated vascular dysfunction.

Study Design: Endothelial-dependent and independent- vascular function of mesenteric arteries was assessed by isometric myograph. Arteries from pregnant female C57/B6J control lean (n=7), control obese (n=12), DDAH1 overexpressing lean (n=6) and DDAH1 obese (n=10) mice were investigated. Obesity was induced by a high fat diet (42% fat adjusted calories, Harlan Teklad) for 8 weeks prior to mating. Data were analyzed by repeated measures ANOVA.

Results: DDAH1 transgenic mice and control wild-type mice both increased body weight compared to lean mice (p<0.01). Obese mice also evidenced elevated leptin (control obese= 4.1ng/ml and DDAH1 obese 3.9ng/ml) compared to lean mice (1.1ng/ml, p<0.05) reflecting increased adiposity. Contractile response to phenylephrine was not different between arteries from all groups of pregnant mice, however tension was greater in arteries from control obese mice compared to control lean mice (p<0.01). Methacholine-induced endothelial-dependent relaxation was significantly blunted in arteries from control obese mice (66±14%) compared to control lean mice (93±2%, p<0.001). In contrast, endothelial-dependent relaxation was intact in arteries from DDAH1 obese mice (90±8%). Endothelial-dependent relaxation was restored in arteries from control obese mice by ex-vivo L-arginine (91±9%). Endothelial-dependent relaxation was not different between arteries from all groups of mice in the presence of the nitric oxide synthase inhibitor L-NAME. Similarly, endothelial-independent relaxation in response to nitroprusside was not different between arteries from all groups of mice.

Conclusion: Overexpression of the ADMA metabolizing enzyme DDAH1 is protective of the obesity-mediated loss in endothelial-dependent relaxation. These data suggest that elevated ADMA is a significant contributor to obesity induced vascular dysfunction in pregnancy.

This project supported by National Institutes of Health grant R01-HL091094.

T-333

Maternal L-Citrulline Supplementation Does Not Affect Blood Pressure, Renal or Vascular Function in Offspring. Robert W Powers, Julia Gefter, Stacy McGonigal, Robin E Gandley. *Magee-Womens Research Institute, Dept OBGYN-RS, University of Pittsburgh, Pittsburgh, PA, USA.*

Context: L-arginine is the nitrogen donating substrate for nitric oxide synthase. Oral L-citrulline is actively metabolized to L-arginine leading to significantly higher L-arginine compared to oral L-arginine. Maternal L-arginine supplementation is associated with improved pregnancy outcomes including decreased prevalence of preeclampsia and IUGR. However, maternal L-citrulline supplementation has been reported to lead to significant hypertension and renal oxidative stress in the offspring.

Objective: The objective of this study was to investigate the effect of maternal L-citrulline administration on changes in blood pressure, renal and vascular function in the offspring.

Study Design: Pregnant female C57/B6J mice were administered L-citrulline (0.25%) in drinking water from day 7 of pregnancy to delivery (n=19), or pregnancy through weaning (n=19) compared to untreated controls (n=11). Blood pressure in offspring was followed to 12 weeks of age. The following variables were measured in all offspring: body weight, heart weight, kidney weight, glomeruli number, area and circumference, plasma L-arginine, ADMA and SDMA, kidney staining for 8-OHdG and nitrotyrosine to assess oxidative stress, endothelial outgrowth from aortic rings, circulating endothelial progenitor cells, endothelial-dependent and independent vascular function in mesenteric arteries by isometric wire myograph and myogenic tone by pressure myograph. The same experiments were also performed in similarly treated pregnant female Sprague Dawley rats.
Results: There were no significant differences in blood pressure in offspring (total or separated by sex) between the different treatment groups (table). In addition, none of the other variables measured were different in the offspring of citrulline treated dams compared to controls.
Conclusion: Contrary to published results, maternal L-citrulline supplementation during pregnancy and lactation in pregnant mice and rats does not affect blood pressure in the offspring.

Blood pressure (mmHg)	4 weeks old	8 weeks old	12 weeks old
Control (n=11)	93.4±8.7/ 67.1±7.5	91.1±8.0/ 66.3±7.2	96.9±9.9/ 70.1±7.6
L-citrulline, pregnancy and lactation (n=19)	102.4±13.7/ 73.3±11.5	92.8±8.3/ 69.4±9.5	98.8±10.3/ 72.5±10.8
L-citrulline, pregnancy only (n=19)	97.2±13.5/ 69.5±11.5	89.4±8.7/ 64.7±8.8	96.1±10.3/ 70.5±8.9

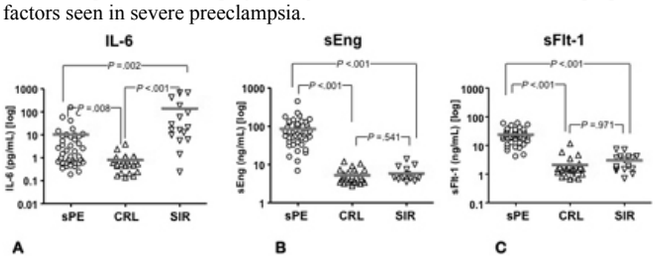
This project supported by National Institutes of Health grant P01-HD30367.

T-334

Overexpression of the Small Conductance Ca^{2+}-Activated K^+ (SK3) Channel Leads to Pregnancy Loss and Altered Expression of Angiogenic Factors. Cara C Rada,[1] Stephanie L Pierce,[2] Noelle C Bowdler,[3] Robert M Weiss,[4] Sarah K England.[1] *[1]Dept. of Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA; [2]Dept. of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA, USA; [3]Dept. of Obstetrics and Gynecology, University of Iowa, Iowa City, IA, USA; [4]Dept. of Internal Medicine, University of Iowa, Iowa City, IA, USA.*
Improper vascularization at the maternal-fetal interface can cause pregnancy complications. Recent evidence indicates that K^+ channels contribute to vascular remodeling during pregnancy and their dysfunction may underlie pregnancy-related vascular diseases. We focused on how pregnancy-dependent vascular remodeling affects pregnancy in a transgenic mouse model overexpressing the small conductance Ca^{2+}-activated K^+ channel ($SK3^{T/T}$). To examine this we used echocardiograms and uterine sonography, quantification of maternal angiogenic soluble factors, and examination of fetal demise rates. The $SK3^{T/T}$ mice exhibit a significant degree of fetal loss with 80% of dams exhibiting some fetal loss when compared to only 28% of the WT dams. Moreover, 51% of the $SK3^{T/T}$ implantation sites end in fetal loss compared to only 8% of WT. Examination of the viable fetuses on pregnancy day 14 (P14) by uterine sonography showed a significant decrease in fetal size when compared to WT mice; thus $SK3^{T/T}$ mice display an intrauterine growth restricted (IUGR) phenotype. The $SK3^{T/T}$ mice also show a decrease in placental thickness. To examine whether the fetal loss and IUGR phenotypes were due to an altered angiogenic state, we measured maternal serum levels of vascular endothelial growth factor (VEGF-proangiogenic) and one of its receptors sFlt-1 (anti-angiogenic) by ELISA. The $SK3^{T/T}$ VEGF levels at P18 were elevated when compared to WT P18 mice, though no difference was seen between non-pregnant (NP) mice. Similarly, sFlt-1 levels in NP WT and $SK3^{T/T}$ mice were the same whereas the levels of sFlt-1 at P18 were significantly decreased in $SK3^{T/T}$ mice as compared to the controls. This data suggests that $SK3^{T/T}$ mice are maintaining a pro-angiogenic state longer, resulting in improper vascularization and fetal loss. Although poor maternal blood flow has been known to cause fetal distress, an enhanced angiogenic state also appears to be maladaptive resulting in increased fetal loss and IUGR. Therefore maintaining an angiogenic balance in pregnancy is important to maintain a healthy full term pregnancy.

T-335

The Elevation in Circulating Anti-Angiogenic Factors Is Independent of Markers of Neutrophil Activation in Preeclampsia. Wenda Ramma,[1] Irina A Buhimschi,[2] Guomao Zhao,[2] Antonette T Dulay,[2] Unzila Ali Nayeri,[2] Catalin S Buhimschi,[2] Asif Ahmed.[1] *[1]University/BHF Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Obstetrics, Gynecology and Reproductive Science, Yale University, New Haven, CT, USA.*
Endothelial cell activation is a hallmark of preeclampsia, which is also associated with neutrophil activation and release of soluble endoglin (sEng) and soluble Flt-1 (sFlt-1) in the maternal circulation. In this study, we investigated the relationships between the circulating markers of neutrophil activation and sEng and sFlt-1 in pregnant women with severe preeclampsia or systemic inflammation without preeclampsia. ELISA was performed for sEng, sFlt-1, placenta growth factor (PlGF), interleukin-6 (IL-6), α-defensins, and calprotectin levels on blood samples of 88 women stratified as follows: severe preeclampsia (sPE, n=45, GA: 30 [27-32 weeks]), maternal systemic inflammatory response (SIR, n=16, GA: 30 [28-33 weeks]) secondary to chorioamnionitis, pyelonephritis or appendicitis; and normotensive controls (CRL, n=27, GA: 29 [26-32 weeks]). Analysis revealed that the levels of α-defensins and calprotectin were two-fold higher in sPE ($p<0.05$) and SIR ($p<0.05$) than CRL indicating increased neutrophil activation. Serum of women with sPE had several fold elevated levels of sFlt-1 ($p<0.001$) and sEng ($p<0.001$) compared to CRL, while women with SIR had sFlt-1 and sEng levels similar to CRL. Highest levels of IL-6 levels were found in SIR cases, although women with sPE also had high levels compared to CRL ($p<0.01$). No correlation between anti-angiogenic factors and the studied markers of neutrophil activation [sEng & α-defensins (P=0.376) and sEng & calprotectin (P=0.133)] or levels of IL-6 [sEng & IL-6 (P=0.597) and sFlt-1 & IL-6 (P=0.423)] were found in sPE. This study demonstrates that despite the association between the anti-angiogenic and inflammatory system in preeclampsia, it is highly unlikely that neutrophil activation plays a major role in the increase in anti-angiogenic factors seen in severe preeclampsia.



T-336

Angiogenic Factors and the Risk of Adverse Outcomes in Twin Gestation. Sarosh Rana,[1] Michele R Hacker,[1] Saira Salahuddin,[2] S Ananth Karumanchi.[1,3,4] *[1]Obstetrics and Gynecology, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston; [2]Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston; [3]Medicine/Nephrology, Beth Israel Deaconess Medical Center/Harvard Medical School and HHMI, Boston.*
Objective
Our objective was to evaluate whether angiogenic factor levels correlate with the diagnosis of preeclampsia (PE) and predict adverse maternal and perinatal outcomes in women with twin gestation.
Study Design
This was a prospective cohort study of women with suspected PE and twin pregnancy from July 2009-October 2010. Antiangiogenic soluble fms-like tyrosine kinase 1 (sFlt1) and proangiogenic placental growth factor (PlGF) at presentation were measured with commercially available ELISA's. Adverse outcomes (severe hypertension; HELLP syndrome; disseminated intravascular coagulation; abruption; pulmonary edema; cerebral hemorrhage; maternal, fetal and neonatal death; eclampsia; acute renal failure; small for gestational age; and indicated delivery) were recorded 2 weeks later. sFlt1/PlGF ratios are reported as median (interquartile range).
Results
There were 55 women with twin gestation. The mean gestational age at enrollment was 33.1±3.6 weeks. The incidence of PE was 60.0%, of which 57.6% was mild and 42.4% was severe. The median sFlt1/PlGF ratio was higher in women with PE [146.6 (87.4-247.8)] compared to women without [66.2 (14.0-137.7); P=0.006]. Women with severe PE had a higher median sFlt1/PlGF ratio [225.5 (146.6-349.9)] than women with mild PE [118.8 (49.4-196.6); P=0.003]. The median sFlt1/PlGF ratio at presentation was

elevated in women who experienced adverse outcomes within 2 weeks [145.9 (79.3-243.6)] compared to those who did not [44.2 (12.5-137.7); P=0.003]. The risk ratio (95% CI) for adverse outcome was 1.9 (1.0-3.6) in the second sFlt1/PlGF tertile compared to the first and 2.1 (1.2-4.0) in the third sFlt1/PlGF tertile compared to the first.

Conclusions

In women with twin gestation and suspected PE, the sFlt1/PlGF ratio is associated with subsequent diagnosis of PE and, more importantly, PE-related adverse maternal and perinatal outcomes. Measurement of angiogenic factors at initial presentation for suspected PE may aid in risk stratification. These findings are similar to singleton pregnancies and may implicate similar pathogenic pathways.

T-337

Chronic Indoleamine 2,3 Dioxygenase Deficiency: A Novel Immunologic Murine Model of Preeclampsia. Mark Santillan,[1] Christopher Pelham,[2] Pimonrat Ketsawatsomkron,[2] Donna Santillan,[1] Baoli Yang,[1] Stephen Hunter,[1] Curt Sigmund.[2] *[1]Obstetrics and Gynecology, University of Iowa Carver College of Medicine, Iowa City, IA, USA; [2]Pharmacology, University of Iowa Carver College of Medicine, Iowa City, IA, USA.*

OBJECTIVE: Current data suggest that immunologic rejection of the feto-placental unit is an important, initiating molecular cause of preeclampsia. Indoleamine 2,3 dioxygenase (IDO) is involved in the maternal tolerance of the allogeneic fetus. Acute IDO inhibition in pregnancy results in fetal demise and hypertension. In humans, lower placental IDO activity is found in preeclamptics. The objective of this study is to test the hypothesis that chronic deficiency of this enzyme in $IDO^{-/-}$ dams is associated with a preeclampsia phenotype.

STUDY DESIGN: $IDO^{-/-}$ mice were generated on a C57Bl/6 background utilizing Cre-Lox recombination. Subsequent loss of IDO mRNA and protein was confirmed by RT-PCR and Western blot. Blood pressure was measured via telemetry. Urine was collected for 24 hours starting at gestational day (GD) 17 for total protein concentration. On GD 18, these syngeneically mated control and $IDO^{-/-}$ dams were sacrificed. Pup numbers and weights were recorded. Vascular reactivity of the aorta and mesenteric vessels were measured *ex vivo* using a wire myograph and a pressurized myograph, respectively.

RESULTS: Absence of the IDO mRNA and protein was confirmed. At GD 18, $IDO^{-/-}$ dams had significantly higher mean arterial pressure (118 vs 107 mmHg, control, $p<0.05$) and proteinuria (4334 vs 2976 mcg/dL, $p<0.001$). Pup number and weight were similar between the groups. Endothelium-mediated relaxation was impaired in aortic rings isolated from $IDO^{-/-}$ dams (43%; 0.1 mM Acetylcholine, $p<0.001$) in comparison to control dams (62%), non-pregnant $IDO^{-/-}$ mice (71%) and control mice (67%). Relaxation to a nitric oxide donor (sodium nitroprusside) was equal between groups. This endothelium specific vascular dysfunction was not observed in mesenteric arteries.

CONCLUSION: The $IDO^{-/-}$ murine model exhibits aspects of the preeclampsia phenotype including elevated proteinuria, higher mean arterial pressure, and *pregnancy induced* endothelial dysfunction. This model, along with acute IDO inhibition models, emphasizes the importance of the role of IDO in the development of preeclampsia. Future studies are underway to understand the molecular vascular biology underlying the endothelial dysfunction and hypertension in this model.

T-338

Vascular Adaptations to 12-Weeks Cycling Training in Formerly Preeclamptic Women. Ralph R Scholten, Dick Thijssen, Fred K Lotgering, Maria TE Hopman, Marc EA Spaanderman. *Obstetrics/Gynecology & Physiology, Radboud University Nijmegen Medical Centre, Netherlands.*

Objective

Formerly preeclamptic women demonstrate arterial changes (increased wall thickness and attenuated endothelial function) and autonomic dysfunction that are associated with cardiovascular disease. Aim of this study was to assess the effects of 12-weeks endurance training in formerly preeclamptic women on artery characteristics and autonomic function.

Methods

Patient-Control intervention study in 24 formerly preeclamptic women (patients) and 20 controls matched for age (32±4 yrs) and interval post partum (7±1 months). Structural characteristics (intima media thickness(IMT)) and functional properties (flow mediated endothelium dependent dilation(FMD)) of 3 large conduit arteries: carotid artery(CA), brachial artery(BA) and superficial femoral artery(SFA) were measured using echo-doppler before and after 12-week cycling training at 70-80% of max. aerobic capacity. We quantified autonomic activity incl. baroreceptor sensitivity(BRS) using spectral analysis of recordings of heart rate variability(HRV).

Results

Patients have increased carotid IMT compared with controls. Training reduced IMT in both groups. At baseline, patients demonstrated reduced endothelial function(FMD). Training improved FMD in both groups. Endothelium independent vasodilation in response to sublingual glyceryl trinitrate did not differ between groups and did not change with training. Physical fitness was relatively low in both groups reflecting sedentary postpartum lifestyles (VO_2max: 27±4 vs.28±4 $mlO_2/kg/min$;p=0.25). Training improved physical fitness in both groups (mean:+15%). HRV-analysis demonstrated increased Low/High Frequency power ratio(LF/HF) in patients indicating sympathetic dominance. Aerobic exercise reduced LF/HF more markedly in patients ($p<0.01$). BRS in patients was evidently reduced (9±4vs.18±6 $ms{\cdot}mmHg^{-1}$;$p<0.01$). Physical exercise improved BRS similarly in both groups.

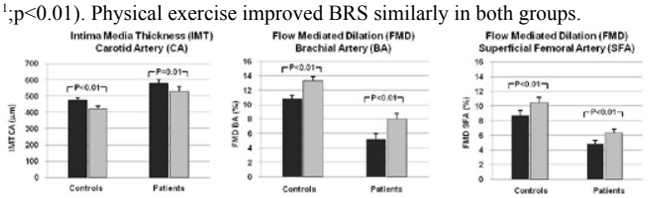


Fig 1. Arterial Characteristics (mean±SEM) in formerly preeclamptic women (patients) and controls before ( ) and after ( ) 12-week cycling training

Conclusion

12 weeks high intensity cycling training improves vascular functions in formerly preeclamptic women and controls. Autonomic nervous system may play an important role in these vascular adaptations.

T-339

Racial Disparity in Maternal Plasma Angiogenic and Anti-Angiogenic Factor Concentration between African Americans and Hispanics: A Longitudinal Study. Eleazar Soto,[1,2] Tinnakorn Chaiworapongsa,[1,2] Zeynep Alpay Savasan,[1,2] Edgar Hernandez-Andrade,[1,2] Lami Yeo,[1,2] Adi Tarca,[1] Zhong Dong,[1] Sonia S Hassan,[1,2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective: An imbalance in angiogenic and anti-angiogenic factors has been demonstrated in preeclampsia, fetal growth restriction, and fetal death. The frequency of these complications vary according to race. The plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), and soluble vascular endothelial growth factor receptor-1 and 2 (sVEGFR-1 and sVEGFR-2) have also been reported to vary between different races in cross-sectional studies. The aim of this study was to compare plasma concentrations of angiogenic/anti-angiogenic factors between African Americans (AA) and Hispanics in a longitudinal study.

Methods: The study included uncomplicated pregnancies from two cohorts of Hispanics (n=150; 903 samples) and AA (n=156; 822 samples). Maternal plasma concentrations of PlGF, sEng, sVEGFR-1 and -2 were determined by ELISA. Linear mixed-effects models were used to fit the log concentration of a given analyte on covariates. To allow for nonlinearity between concentration and GA, age was split into 4 intervals, with the 3 cut-points being the 0.25, 0.5, and 0.75 quartiles of the distribution of all GA values. Model fitting and significance of fixed effects was calculated.

Results: 1) the mean plasma concentration of the pro-angiogenic factor, PlGF, in Hispanics was lower than that of AA before 18 weeks. This became more pronounced after 31 weeks; 2) the mean plasma concentration of the anti-angiogenic factor, sEng, was higher in Hispanics than AA in early gestation, and the difference was even more pronounced after 26 and 31 weeks; and 3) the mean plasma concentrations of sVEGFR-1 and -2 were different between the 2 cohorts after 31 weeks.

	GA (weeks)			
	8-18	18-26	26-31	31-39
PlGF	↓*	↔	↔	↓*
Endoglin	↑*	↔	↑*	↑*
sVEGFR-1	↔	↔	↔	↑*
sVEGFR-2	↔	↔	↔	↓*
PlGF/sVEGFR-1	↓*	↔	↔	↓*
PlGF/sEng	↓*	↔	↓*	↓*

* $p<0.05$; arrows represent maternal plasma mean difference in Hispanics compared to African Americans

Conclusions: Hispanics have different plasma concentrations of angiogenic and anti-angiogenic factors from AA, favoring an anti-angiogenic profile in the former. Assessment of the concentration of these factors needs to take race into account.

T-340

Do Circulating Angiogenic Factors Predict Preeclampsia among Twins?
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Objective: Pathophysiologic events leading to preeclampsia (PE) may begin in the first or early second trimester. Studies have shown aberrations in angiogenic factors preceding clinical signs of PE, and twins are at increased risk of PE. We investigated if sequential maternal concentrations of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PLGF) can predict PE among twin gestations.

Methods: Prospective cohort of women with twins receiving prenatal care at one of three tertiary care academic centers. Women completed surveys on demographics, and relevant medical information was abstracted from records. Maternal blood and urine samples were collected at four visits, and plasma sFlt-1 and PLGF concentrations were quantified by specific immunoassay. Wilcoxon Rank Sum test compared medians of sFlt-1, PLGF, and the PLGF/s-Flt-1 ratio. Optimal cutoffs for the analytes were calculated with receiver operating characteristic curves, and relative risk (RR) of PE as a function of high versus low analyte concentration based on optimal cutoffs for each analyte was estimated. Assuming a 10% incidence of PE among twins, power exceeded 0.80.

Results: 121 twin gestations were enrolled from 2007-2009. At approximately 11, 18, 26, and 35 weeks, no differences were observed in concentrations of sFlt-1, PLGF, or PLGF/sFlt-1 comparing uncomplicated pregnancies to those with PE. Additionally, the RR of PE for each of the analytes at each time interval was not significant, although the RR of PE based on sFlt-1 concentrations at 35 weeks approached statistical significance.

Median Weeks	Analyte	Optimum Cutoff	N	RR (95% CI)
10	PLGF	≤24.3	119	1.30 [0.73–2.34]
	sFlt-1	≥9.3	119	1.01 [0.57–1.81]
	PLGF/sFlt-1	≤3.0	119	0.93 [0.52–1.65]
17	PLGF	≤218.2	115	0.98 [0.55–1.77]
	sFlt-1	≥12.5	115	1.02 [0.56–1.83]
	PLGF/sFlt-1	≤16.5	115	0.90 [0.50–1.62]
24	PLGF	≤642.0	118	0.91 [0.50–1.65]
	sFlt-1	≥14.2	118	1.42 [0.78–2.59]
	PLGF/sFlt-1	≤42.0	118	1.30 [0.72–2.34]
35	PLGF	≤230.7	83	1.67 [0.68–1.09]
	sFlt-1	≥37.6	83	2.24 [0.98–5.11]
	PLGF/sFlt-1	≤6.2	83	1.48 [0.66–3.30]

Conclusions: Sequential sFlt-1 and PLGF concentrations exhibited poor performance for predicting PE. Aberrations in angiogenic concentrations among singletons may not translate to multiple gestations, and further investigations should focus on other potential markers of PE for twins.

T-341

Differential VEGF mRNA Splicing in Preeclampsia and Normotensive Human Placentas.
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Introduction: Vascular endothelial growth factor (VEGF) is indispensable for regulating placental angiogenesis. In addition to the normal VEGF splice variants (121, 145, 165, 189 and 346), recent studies have shown that alternative splicing at a distal site in the terminal exon 8 of *VEGF* results in the formation of novel VEGF isoforms ($VEGF_{xxx}b$) that translate proteins of the same length as the normal VEGF isoforms, but with different sequence and anti-angiogenic property. However, whether these alternative *VEGF* splice variants are differentially expressed in preeclampsia is not fully understood. **Objectives**: to evaluate the differential expression of VEGF mRNA splicing variants in preeclampsia and normotensive human placentas. **Methods**: Placentas from normotensive (n=7) and severe preeclamptic (n=10) patients were obtained at delivery. Total RNA samples were extracted using Trizol reagent and 2 µg/sample were reversed transcribed. Polymerase chain reaction (PCR) was performed by using a forward primer (exon 4, 5'-GAGATGAGCTTCCTACAGCAC-3') with a reverse primer (exon 8a, 5'-CTCACCGCCTCGGCTTGTCAC-3) for amplifying the normal VEGF isoforms, whereas the alternative $VEGF_{xxx}b$ splice variants were amplified by using the same forward primer with a reverse primer (8b, 5'-TCAGTCTTTCCTGGTGAGAGATCTGCA-3). The amplicons were analyzed by agarose gel electrophoresis and sequencing. **Results**: The normal VEGF isoforms amplified with the exon 8a primer (amplicons of 346 bp, 295 bp, 188 bp, and 91 bp, respectively) were detected in both normal and pre-eclamptic placentas. The $VEGF_{165}b$ mRNAs was detected in both normal (67%) and pre-eclamptic placentas (60%) whereas $VEGF_{121}b$ was only detected in preeclamptic samples. **Conclusions**: VEGF mRNA splicing is differentially regulated in normotensive and preeclamptic placentas. Specific preeclamptic placental $VEGF_{121}b$ expression implicates that alternative VEGF splicing is a crucial mechanism for placental angiogenesis that is deranged in preeclampsia (Supported by NIH RO1 HL70562 & R21 HL98746).

T-342

Haptoglobin Phenotype, Angiogenic Factors and Preeclampsia Risk.
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Objective: The hemoglobin-binding protein haptoglobin (Hp) is a powerful anti-oxidant and angiogenic factor. The 3 common polymorphisms (1-1, 2-1, 2-2) differ in structure and function. Hp 1-1 is the strongest anti-oxidant. Hp 2-2 is the most angiogenic. The study objective was to determine whether Hp phenotype is related to preeclampsia risk, or to plasma concentrations of soluble endoglin (sEng), soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF).

Methods: Hp phenotype was determined in primiparous women with uncomplicated pregnancies (n=309), gestational hypertension (n=215) and preeclampsia (n=249). Phenotype was assessed by peroxidase staining following native polyacrylamide gel electrophoresis of hemoglobin-supplemented serum. Angiogenic factors were measured in preeclamptic women (n=77) and controls (n=247) matched for gestational age. Phenotypes were compared by logistic regression.

Results: Compared to Hp 1-1, Hp 2-1 was associated with a significantly increased risk of preeclampsia and term preeclampsia in Caucasians. Hp phenotype was not associated with preeclampsia risk in African Americans, or with the risk of gestational hypertension in either race.

Table 1: Odds ratios for preeclampsia and gestational hypertension, relative to Hp 1-1

Outcome	Race	Hp 2-1 OR (95% CI)	p	Hp 2-2 OR (95% CI)	p
Preeclampsia	Caucasian	2.11 (1.07, 4.18)	0.019	1.47 (0.74, 2.92)	0.960
	African American	1.25 (0.59, 2.64)	0.811	1.33 (0.54, 3.27)	0.657
Gestational Hypertension	Caucasian	1.23 (0.64, 2.38)	0.608	1.19 (0.62, 2.30)	0.761
	African American	1.41 (0.65, 3.07)	0.464	1.23 (0.47, 3.17)	0.939

Abbreviations: OR, odds ratio; CI, confidence interval. Adjusted for age.

Table 2: Odds ratios for term and preterm preeclampsia in Caucasians, relative to Hp 1-1

Preeclampsia Type	Hp 2-1 OR(95% CI)	p	Hp 2-2 OR(95% CI)	p
Preterm	1.87 (0.79, 4.39)	0.084	1.24 (0.52, 2.94)	0.751
Term	2.45 (1.07, 5.83)	0.025	1.73 (0.74, 4.05)	0.757

Adjusted for age.

Preeclamptic women had higher plasma sEng and sFlt-1, and lower PlGF, than controls. sEng, sFlt-1 and PlGF did not differ by Hp phenotype.

Conclusion: Hp 2-1 is associated with higher preeclampsia risk in primiparous Caucasian women. Angiogenic factors were not related to Hp phenotype.

T-343

Risk of Abnormal Uterine Artery Doppler According to Haptoglobin Phenotype.
Tracey L Weissgerber. *for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network, Bethesda, MD.*

Objective: Abnormal uterine artery Doppler velocimetry (UADV) is thought to reflect high uterine vascular resistance. Impaired angiogenesis and oxidative stress contribute to abnormal vascular remodeling and placentation, and subsequently to adverse pregnancy outcomes. The hemoglobin-binding protein haptoglobin (Hp) is a powerful anti-oxidant, and pro-angiogenic factor. Hp is involved in implantation, and concentrations in the uterine decidua increase during pregnancy. Hp has four structurally and functionally distinct polymorphisms (1-1, 2-1, 2-1M and 2-2). Hp 1-1 is the strongest anti-oxidant, whereas Hp 2-2 is the strongest angiogenic factor. The objective of this study was to determine whether Hp phenotype is associated with abnormal mid-trimester UADV.

Methods: UADV was assessed at 13-20 (median 16.6) weeks gestation in 2,184 nulliparous, low risk pregnant women who were randomized to

receive daily vitamin C (1000 mg) and E (400 IU), or placebo, beginning at 9-12 weeks gestation. Waveforms were evaluated to determine the resistance index, pulsatility index, and the presence of any notching or bilateral notching. Hp phenotype was determined by peroxidase staining following native polyacrylamide gel electrophoresis of hemoglobin-supplemented serum. Hp phenotypes were compared using multivariable logistic regression and generalized linear models.

Results: There was no relationship between Hp phenotype and the resistance index, pulsatility index, or the presence of any notching or bilateral notching. Analyses were adjusted for treatment group, variables that were associated with abnormal UADV (gestational age at Doppler exam, body weight) and variables that differed between women of different phenotypes (age, race, years of schooling, pre-randomization prenatal vitamin use) (Table 1).

Table 1: UADV Indices According to Hp Phenotype

Outcome	Hp 1-1 (n=449)	Hp 2-1 (n=1025)	Hp 2-2 (n=661)	Hp 2-1M (n=49)	p[a]
Any Notch	1.31 (0.92, 1.85)	1.21 (0.91, 1.60)	1	1.39 (0.61, 3.21)	0.42
Bilateral Notch	1.19 (0.70, 2.01)	1.19 (0.78, 1.82)	1	1.04 (0.29, 3.72)	0.87
Resistance Index	0.619	0.623	0.614	0.629	0.19
Pulsatility Index	1.18	1.20	1.20	1.18	0.71

Values are adjusted odds ratio (95% confidence interval) or adjusted mean. [a]Adjusted for confounding variables.

Conclusion: Hp phenotype is not associated with the risk of abnormal uterine artery Doppler.

T-344

Expression of G-Protein Subunit-α11 and 14 in Human Placentas. Yingjie Zhao, Yan Li, Huihui Li, Kai Wang, Jing Zheng. *Dept. of Ob/Gyn, Univ. of Wisconsin, Madison, WI, USA.*

Background: During pregnancy, dramatic vascular growth in the fetus and placenta is critical for the remarkably increased fetal and placental blood flows required for supporting the developing fetus. G-protein coupled receptors represent by far the largest family of cell-surface molecules, which mediate numerous cell functions upon interacting with G-proteins. Their dysfunction contributes to some of the most prevalent human diseases such as hypertension and cancers. G-protein subunit α-11 (GNA11) and 14 (GNA14) are two members in the Gα family, which as transducers are involved in various transmembrane signaling including PLC and Ca++, key signaling molecules for endothelial functions. GNA11 has been implicated in modulating VEGF's signaling in endothelial cells; however, little is known about roles of GNA14 in any placental cell functions. We have found that physiological chronic hypoxia significantly enhanced VEGF- and FGF2-stimulated cell proliferation and migration, which are associated with robustly increases in mRNA of GNA14, but not GNA11 in human umbilical cord vein (HUVE) and artery (HUAE) endothelial cells. **Methods:** To explore potential roles of GNA11 and GNA14 in human placentas, in this study, we examined GNA14 and GNA11 protein expression in human placentas obtained from first and third trimester pregnancies as well as from normal (N) and severe preeclamptic (sPE) pregnancies. GNA14 protein expression was also determined in (HUVE and HUAE cells cultured under chronic normoxia (~ 20% O_2) and hypoxia (3% O_2) using Western blotting. **Results**: 1) No significant difference in GNA11 and GNA14 was found in in placentas from the first trimester vs. the third trimester pregnancies; 2) The levels of GNA14, but not GNA11 protein in sPE placentas were increased ~ 2.94 fold ($p \leq 0.01$) in sPE vs. N placentas; and 3). Physiological chronic hypoxia (3% O_2) significantly promoted GNA14 (~ 1.63 fold, $p \leq 0.05$), but not GNA11 protein expression in HUVE, but not HUAE cells. **Conclusions:** Our data suggest that GNA14 may play an important role in placental and fetal vascular endothelial functions, especially under chronic hypoxia and in sPE pregnancies.

T-345

Estrogenic Potential of Androstenediol. Sara R Pittenger,[1] Marcelle I Cedars,[1] Daniel S McConnell,[2] Robert Handa,[3] Ryoko Hiroi,[3] Jiangang Chen,[5] Nancy Gee,[4,6] Bill L Lasley.[4] *[1]Obstetrics and Gynecology, University of California, San Francisco, CA, USA; [2]Epidemiology, University of Michigan, Ann Arbor, MI, USA; [3]College of Medicine, University of Arizona, Phoenix, AZ, USA; [4]Center for Health and the Environment, University of California, Davis, CA, USA; [5]Public Health, University of Tennessee, Knoxville, TN, USA; [6]California National Primate Research Center, University of California, Davis, CA, USA.*

Background: Nearly 85% of women have increases in circulating adrenal delta five steroids during the menopausal transition (MT). One of these steroids, androstenediol (Adiol) ranges in concentration from 100 to 1,000 pg/mL at or near the MT, while circulating estradiol (E2) levels decline to 10 pg/mL or less. Adiol has both androgenic and estrogenic properties and activates estrogen receptor (ER)-alpha and -beta in vitro. In the physiological environment of declining E2, the relatively higher circulating levels of Adiol may have a greater effect on estrogen-sensitive tissues than previously considered.

Aim: To characterize Adiol activity through ER-alpha and -beta receptors.

Methods: Two cell lines were prepared by transfecting ovarian carcinoma BG1 cells with ER-alpha and hypothalamic N38 cells with ER-beta. Both cell lines were transfected with a luciferase reporter gene driven by a promoter containing multiple estrogen response elements. Increasing doses of E2, Adiol, or a combination of the two were added and luciferase activity was measured.

Results: Adiol's estrogenic potential through ER-alpha is approximately 0.01% that of E2. With the addition of E2, higher levels of Adiol augment the transcriptional activity of E2. The ER-beta potential of Adiol is 1.0% that of E2. Adiol in combination with E2 acts to decrease ER-beta mediated activity.

Conclusion: While E2 levels change relatively little during the MT, Adiol has a ten-fold range of circulating concentrations between women. This change in adrenal steroids may better explain the individual differences in estrogen-related symptomatology at the time of the MT than changes in E2 alone. In addition, the pure agonistic effect of Adiol through the ER-alpha signaling pathway and the agonist/antagonist effect through the ER-beta signaling pathway may indicate that Adiol has specific effects in different estrogen-sensitive tissues depending on the predominance of ER types and the E2 levels.

Support: MH082679, NS039951, P51 RR00169, P42 ES04699

T-346

The Effect of Paternal Age on Outcome in Assisted Reproductive Technology Using the Ovum Donation Model. Isela M Robertshaw,[1] Glen E Hofmann,[1] Mazen E Abdallah,[1] Paradeep Warikoo,[1] Jane Khoury.[2] *[1]Obstetrics and Gynecology, Division of Reproductive Endocrenology and Infertility, Cincinnati, OH, USA; [2]Obstetrics and Gynecology, Division of Biostatistics, Children's Hospital, Cincinnati, OH, USA.*

Objective: To determine the effect of paternal age on live birth rates in assisted reproductive technology (ART) using the ovum donation model.

Design: Retrospective database review conducted with IRB approval.

Materials and Methods: The ovum donation model provides an opportunity to analyze the paternal age impact on ART outcome, given that the ovum is invariably of superior quality. 237 consecutive donor ovum IVF cycles performed at our center were reviewed. Donors were stimulated with GnRHa down regulation and hMG. All recipients were prepared with GnRHa down regulation and estrogen and progesterone replacement cycles. All embryo transfers were done at blastocyst stage under ultrasound guidance. The impact of the paternal age on outcome was analyzed controlling for number and grade of embryos transferred. The ART outcome was divided into three groups: no pregnancy, miscarriage, and ongoing/live births.

Results: Out of the 237 cycles, 36 resulted in no pregnancy, 31 ended in miscarriage and 170 cycles resulted in ongoing pregnancies (8) or live births (162). The mean paternal age (MPA) was significantly different among the 3 pregnancy outcome groups: non pregnant (MPA 42.6 ± 8.4), miscarriage (MPA 41.6 ± 5.9), and pregnant (MPA 39.3 ± 6.5), $p = 0.01$. The mean number and grade of embryos transferred were 2.1 ± 0.4 and 1.3 ± 0.3 respectively. There was no difference in the number or grade of embryos transferred among the three different pregnancy outcome groups. Of the 237 cycles, 49 had no gestational sac (GS) established on ultrasound, 94 had one GS and another 94 had 2 GS with a MPA of 42.7 ± 7.8, 40.1 ± 7.1 and 39.1 ± 5.7, respectively, ($p = 0.01$).

Conclusions: Our data shows that advanced paternal age may have an adverse impact on ART outcome. With similar numbers of embryos transferred, a younger paternal age seems to have a favorable effect on implantation and live birth rate.

T-347

Falsely Elevated FSH Levels in a Human Anti-Mouse Antibodies Positive Patient Who Conceived Following IVF Treatment. Tomer Singer, Jason Kofinas, Jack Y Huang, Hung-Ching Liu, Owen Davis, Zev Rosenwaks. *The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA.*

PURPOSE: To report a case of heterophile antibodies, which included Human anti-mouse antibodies (HAMA), interfering with laboratory hormonal assays.

SETTING: Academic medical center-based IVF practice.

RESULTS:

35 yo G3P0030 white female presented with 2 years of secondary infertility. She was healthy, 5'4'', BMI of 19 and denied immunoglobulin therapy or

animal exposure. Menarche at 13, with regular 30 days menses and 4 flow days. History significant for PID, miscarriage, spontaneously resolving tubal pregnancy and a biochemical pregnancy.
Day-3 FSH was 9.4 IU/L and an HSG revealed loculation of the distal right tube. The patient underwent (abroad) 3 failed Clomiphene citrate cycles, one with IUI. At 35 with presumed tubal factor infertility, she was treated in our center with 1 successful IVF cycle which resulted in an uneventful full term delivery. Two years later (at 38) her FSH levels were found to be 42 IU/L. Repeat FSH levels remained elevated (29-37 IU/L).
An interference with the FSH assay causing falsely elevated FSH levels was suspected. The samples were reviewed and the patient was found to be positive for HAMA. Repeat AMH levels were found normal 1.55-3.6 ng/ml. FSH levels while controlling for HAMA were 1.22 and 2.33 IU/l.
HAMA was only detected in 1 serum sample, which suggested that other interfering human endogenous antibodies might be involved. The block percentage was very high (90-95%).
The patient proceeded with IVF treatment and following the retrieval of 24 oocytes and the transfer of 3 embryos she recently conceived a singleton and had normal first trimester screening.
CONCLUSIONS: This case highlights the importance of maintaining a high index of suspicion for the presence of heterophile antibody interference.
One explanation for the abnormal FSH serum levels in this patient could be the development of anti-FSH antibodies following her IVF treatment.
Inaccuracies in laboratory assays could lead to adverse patient outcomes, delayed diagnosis and delayed treatment.

T-348

Production of Functional Platelets *Via* Megakaryocytes Generated from Human Endometrial Stromal Progenitor Cells. Jinju Wang,[1] Shuzhen Chen,[1] Wenfeng Zhang,[1] Teresa Pfaff-Amesse,[3] Barbara Hull,[2] Yanfang Chen,[1] Lawrence Amesse.[3] *[1]Pharmacology & Toxicology, WSU; [2]Biological Sciences & Internal Medicine, WSU; [3]Obstetrics & Gynecology, Wright State University Boonshoft School of Medicine, Dayton, OH, USA.*
Introduction: Human endometrium is a highly dynamic tissue that contains abundant stem/progenitor cells. Endometrial stromal progenitor cells (hESCs) have been shown to differentiate into mesodermal lineages including fat and chondrocytes. No information exists on differentiating hESCs into hematopoietic cell lines. The aim of this work was to investigate the possibility of differentiating hESCs into the myelocyte lineage with the subsequent production of MKs and functional platelets.
Methods: hESCs were obtained by culturing human endometrial cells for 4-6 passages, and purity was confirmed by flow cytometry. Differentiation into Megakaryocyte (MK) was achieved by culturing hESCs (1.8x105 cells/well) for 18days in serum free media containing 50ng/ml of thrombopoietin (TPO). Derived MKs were analyzed by flow cytometry and confocal microscopy. Platelets were subsequently collected days10-18 from the MK media supernatant, and functional studies for platelet specific markers were performed.
Results: Cultured hESC were highly positive (>95%) for the stromal cell markers CD29 and CD90, and negative (<1.3%) for hematopoietic cell markers CD 34 and CD 45 by flow cytometry. Generation of MKs obtained by culturing these cells inTPO was confirmed by phenotypic expression of the MK membrane markers CD41a: 39 ± 3.0% vs con. 1 ± 0.09% and CD42b: 28 ± 2.0% vs con. 1.2 ± 0.06%, n=3. Immunocytochemistry analysis showed differentiation rate into MK of 38 ± 3.0% with CD41a and of 27 ± 2.5% of CD42b. Generated platelets were positively labeled with CD41a (90 ± 2%). Functional studies on the platelets showed that thrombin (5U/ml) stimulation up-regulated CD62P expression (26.0 ± 4% vs con. 2.5 ± 1%, n=3), and increased fibrinogen binding (32 ± 3.0% vs con. 1 ± 0.4%, n=3) indicating functional integrity of the hESC derived platelets. Electronic microscopic examination showed that the hESC derived and peripheral blood platelets had a similar ultrastructure profile complete with normal storage granules.
Conclusion: It is possible to generate MKs and platelets from hESC. The derived and native platelets were structurally and functionally identical and indicate that hESCs are a potential source of platelet for stem cell-based therapies.

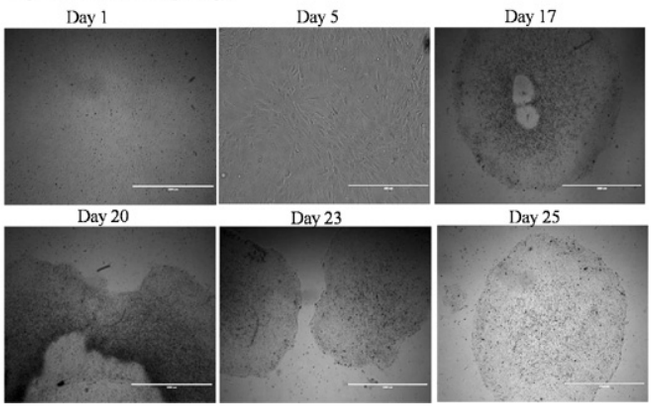
T-349

Endometrial Mesenchymal Stem Cells Differentiate to Endometrial Stromal Fibroblasts and Decidualize in Response to Progesterone. F Barragan, JS Tamaresis, JC Irwin, LC Giudice. *Obstetrics Gynecology and Reproductive Sciences, University of California, San Francisco, CA, USA.*
Background: Adult human endometrium undergoes cyclic shedding and subsequent regeneration that likely involves stem/progenitor cells. Endometrial mesenchymal stem cells (eMSC) co-expressing CD146 and PDGRB surface markers are clonogenic multipotent pericytes that differentiate into non-endometrial mesenchymal cell lineages. A physiological role of eMSC as progenitors of endometrial-specific cell lineages is not well defined. Herein, we investigated the differentiation potential of eMSC to the endometrial stromal fibroblast (eSF) lineage.
Methods: Endometrial tissues were procured through the NIH UCSF Human Endometrial Tissue Bank. Endometrial stromal cells were obtained by enzymatic dissociation and CD146+/PDGFRB+ (eMSC) and CD146-/PDGFRB+ (eSF) populations isolated by fluorescence activated cell sorting (FACS). Primary colonies from FACS isolated eMSC were established in eSF growth medium (eSF-GM: DMEM/MCDB-105+10% FBS and insulin). To assess decidualization colonies were subcultured and grown for 14 days in eSF-GM without additives or with 3-isobutyl-1-methylxanthine (IBMX), known to influence MSC differentiation. Subsequently, cultures were treated with 10nM estradiol+1μM progesterone (E_2P_4) or ethanol vehicle for 14 days in serum- and insulin-free eSF-GM containing BSA, ascorbic acid, transferrin, and EGF. Decidualization was assessed by IGFBP-1 secretion into the conditioned medium assayed by ELISA. Data was analyzed by 3-way ANOVA. Subject-paired primary clonal cultures derived from FACS isolated eSF served as positive controls.
Results: Primary eMSC clones displayed polygonal cell morphology typical of eSF, suggesting differentiation to the eSF lineage. eMSC-derived cultures decidualized in response to P_4, a unique property of eSF, secreting substantial amounts of IGFBP-1 as early as 9 days after starting E_2P_4 treatment, comparable ($p>0.05$) to E_2P_4-treated eSF controls (all averaging 155-159 ng/ml on day 9). IGFBP-1 was below or at detection level (1 ng/ml) in all vehicle treated cultures. Pre-treatment with IBMX did not affect IGFBP-1 levels in any of the E_2P_4-treated groups ($p>0.05$).
Conclusion: Our findings support a role for eMSC as the endometrial progenitor of the endometrial stromal fibroblast lineage.
Support: The *Eunice Kennedy Shriver* NICHD/NIH Specialized Cooperative Centers Program in Reproduction and Infertility Research U54HD 055764-05 (LCG)

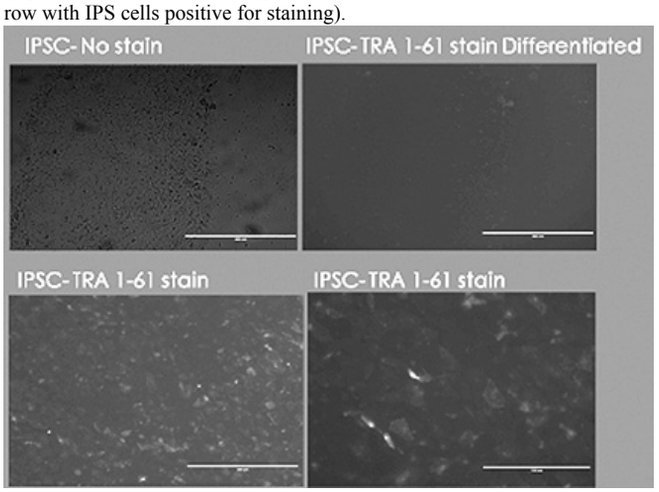
T-350

Development of Pleuripotent Stem Cells (PSC) from Mesenchymal Derived Cells Obtained during Routine Amniocentesis. Jay M Bolnick,[1] Eric R Secor,[2] Michael P Diamond,[1] Xiaonan Xin,[3] Peter Benn,[4] Alex Lichtler.[3] *[1]Division of Reproductive, Endocrinology and Infertility, Wayne State University, Detroit, MI, USA; [2]Department of Immunology, University of Connecticut Health Center, Farmington, CT, USA; [3]Department of Reconstructive Sciences, School of Dental Medicine/University of Connecticut Health Center, Farmington, CT, USA; [4]Department of Ob/Gyn and Maternal Fetal Medicine, University of Connecticut Health Sciences, Farmington, CT, USA.*
Objective: To obtain mesenchymal cells from amniotic fluid and reprogram them into pluripotent stem cells (PSC).
Methods: Mesenchymal cells (MC) were obtained during second trimester amniocentesis (under IRB approval). Cells were cultured and mesenchymal cellular phenotype was confimed via flow cytometry. Cells were than transfected with a Lentiviral vector system to reprogram MC into PSC. Cell transformation into PFC was confirmed via microscopy to evaluate cell morphology and imunocytochemistry staining was used to identify expression of Tumor Rejection Antigen (TRA) 1-61 which is a unique PSC marker.
Results: Mesenchymal stem cells were positive for CD73 and CD44 via flow cytometry. Cell morphology started out as individual fibroblastic looking cells on day 1; to PSC which appeared as rounded,having demarcated edges and tightly packed cell colonies each with scant cytoplasm (day-25).

Figure 1. Cellular morphology



Identification of PSC was confirmed with positive cell staining for TRA 1-61 (top row represents no staining with differentiated control cells and bottom row with IPS cells positive for staining).



Conclusion: This study demonstrates PSC can be successfully generated from fluid obtained during routine amniocentesis. This represents an excellent source of stem cells for use in developing novel treatments for personalized medicine.

T-351

The Effect of Maternal CD34$^+$ HSCs on Suppressed NK Cell Activity in the Postpartum. Nagwa El-Badri,[1] Maureen Groer,[1] Julie Djeu,[2] Bradley Kane,[1] Shaunte Williams,[1] Monalisa Harrington.[1] *[1]Ob/Gyn, University of South Florida, Tampa, FL, USA; [2]Nursing, USF; [3]Cancer Immunology, Moffitt Cancer Center, Tampa, FL, USA; [4]Nursing, USF; [5]Nursing, USF; [6]Nursing, USF.*

BACKGROUND

Fetal microchimerism is defined as the presence of fetal cells in maternal blood and tissues during pregnancy and the post partum. These cells can be found in pregnant and multiparous women, but not in non pregnant women or in men. While the identity and function of microchimeric cells (MC) is poorly defined, an increase in maternal CD34$^+$ cell population in the post partum may play a role in immune modulation after child birth.

METHODS AND RESULTS

In this study, we test the hypothesis that maternal hematopoietic CD 34$^+$ stem cells have a role in the recovery of Natural Killer (NK) cell cytotoxic function in the post partum. We collected blood from 17 women in the post partum period, and from pregnant women. We report an increase in CD34$^+$ cell population in pregnant women and post partum women. A subpopulation of these cells expressed CD117 marker plus specific embryonic markers: SEEA1 and Oct 4. In the post partum, we found that NK cells regularly showed lower numbers and expressed suppressed cytotoxicity. Co-culture of maternal CD34$^+$ cells with NK cells stimulated NK cell cytotoxix activity and increased lytic units. This increase was IL-2 dependent.

CONCLUSIONS

These results identify increase in CD34$^+$ cells in the maternal blood during pregnancy and the post partum. A distinct subpopulation of these cells expresses embryonic markers, Oct4 and SSEA-1 markers. These CD34$^+$ stem cells stimulate NK cell cytotoxicity and may be necessary for recovery of NK lytic cell function in the post partum period.

T-352

Oogonial Stem Cells Increase in Numbers in Aged Mouse Ovaries. Luciana R Faustino, Dori C Woods, Yvonne AR White, Jonathan L Tilly. *Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Bosotn, MA, USA.*

Introduction: while it is well established that ovarian aging is associated with follicle loss, traditional beliefs are that this occurs solely through depletion of a finite pool of oocytes set down at birth. However, the recent identification of female germline or oogonial stem cells (OSCs) in adult mouse ovaries [Nature 2004 428:145], along with evidence that these cells generate fully functional eggs [Nat Cell Biol 2009 11:631], raises the possibility that multiple mechanisms underlie exhaustion of the ovarian reserve with age. In support of this, aged mouse ovaries lacking oocytes possess pre-meiotic germ cells that can generate oocytes if moved to a young adult ovarian environment [Aging 2009 1:971].

Objective: to characterize OSCs isolated from young and aged adult mouse ovaries.

Methods: C57BL/6 mice at 3 (young adult) and 20 (aged adult) months of age were used. Yield of viable OSCs per ovary was assessed by fluorescence activated cell sorting (FACS) using an antibody targeting the extracellular domain of VASA [Nat Cell Biol 2009 11:631]. Identity of OSCs was confirmed by analysis of germline gene expression in freshly isolated cells and in-vitro expanded cells, as well as by the ability of these cells to spontaneously form oocytes in vitro [Differentiation 2010 79:159].

Results: FACS analysis revealed a 6-fold increase in yield of cells with externally-exposed VASA from ovaries of 20-month-old versus 3-month-old mice. PCR analysis confirmed their identity as OSCs, as the cells obtained following FACS were positive for the germline specification genes, Blimp1, Stella, Fragilis and Tert. In addition, the cells could be established in culture and expanded in numbers while maintaining their germline identity. Oocytes spontaneously generated following OSC expansion in vitro were positive for multiple oocyte markers, including Nobox, Sohlh1, Zp3, Gdf9 and c-Kit.

Conclusions: these findings indicate that atrophic aged ovaries lacking oocytes retain OSCs, likely in a quiescent state. Taken with recent findings that the ovarian microenvironment plays a critical role in determining whether OSCs can generate new oocytes to form follicles [Aging 2009 1:971], it is likely that aging-related changes in somatic compartments in the ovary that support OSC function are critical determinants of the inability of these cells to maintain oocyte numbers with increasing age.

Support: NIH R37-AG012279

T-353

Hypoxia Alters Gene Expression of NF-κB-Regulated Genes in Human Placental Mesenchymal Stem Cells. Diharah Fernando, Jennifer M Ryan, Marloes Dekker Nitert, Gregory E Rice, Murray D Mitchell. *UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia.*

Introduction: During early pregnancy (<12 weeks), a low oxygen tension (~1%) is requisite for effective trophoblast invasion of the decidua and establishing materno-fetal circulation. Poor trophoblast invasion is associated with subsequent adverse pregnancy consequences, including pre-eclampsia, IUGR, and preterm birth. Oxygen tension changes from 1% to 8% once contact with the maternal circulation is established. Mesenchymal Stem Cells (MSC) are abundant in the placenta and are more sensitive to hypoxia compared to other resident placental cells (*e.g.* trophoblast). There is, however, a paucity of data concerning the role MSC play in placental development and function. We, therefore, assessed the effects of oxygen tension on placental MSC. In particular, the effects of oxygen tension on mediators of inflammation (i.e. NF-κB regulated genes) were assessed.

Objective: To determine whether sustained changes in oxygen tension affect the expression of NFκB responsive genes.

Methods: MSC were isolated from first trimester placental villi from three different donors. The cells were exposed to two oxygen tensions (1 and 8% O_2) by incubating them in a Biospherix Hypoxia Chamber for a period of seventeen days. Group 1 MSC (n=3) were maintained in sustained oxygen tensions of 8% (physiological normoxia during development) for the duration of the incubation period. Group 2 MSC (n=3) were preconditioned in 8% oxygen tension for two weeks, then transiently exposed to 1% oxygen tension for 24h and returned to 8% oxygen for an additional 24h. The expression levels of NF-κB responsive genes including COX-2, ICAM-1, TNF-α, IL-6 and IL-1β were then determined by real-time PCR.

Results: Group 2 MSC showed a decrease in NF-κB responsive genes compared to cells cultured constantly compared to group 1. The candidate gene included: IL-6 = 16.5%; TNF-α = 11.8%; IL-8 = 8.5%; ICAM-1 = 18.8%; COX-2 = 8.9%).
Discussion: The data obtained in this study are consistent with the hypothesis that transient hypoxic episodes may lead to sustained alteration in the expression of placental MSC inflammatory mediators. The role of inflammatory mediators in implantation and placentation is well established, however, the contribution of placental MSC remains to be clearly elucidated. The hypoxia induced changes observed in this study may contribute to the risk of subsequent complications of pregnancy.

T-354

Characterization of Meiosis in Ovarian Germ Cells of Reproductive-Age Women. Jolijn W Groeneweg,[1] Dori C Woods,[1] Yvonne AR White,[1] Yasushi Takai,[2] Jonathan L Tilly.[1] *[1]Vincent Center for Reproductive Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA; [2]Department of Obstetrics and Gynecology, Saitama Medical Center, Saitama, Japan.*
Introduction: accumulating evidence supports the existence of germline or oogonial stem cells (OSCs) in adult mouse ovaries [Nature 2004 428:145] that can be isolated for in-depth study [Nat Cell Biol 2009 11:631]. We have found that human ovarian cortex contains a comparable population of germ cells that proliferate in vitro and produce oocytes in vitro and in xenografts in vivo. However, the meiotic capacity of these cells remains Yasushi to be determined.
Objective: to study meiosis in adult human ovaries and purified human OSCs.
Methods: as previously reported in mice [Nat Cell Biol 2009 11:631], immunological detection of cell-surface expression of VASA can be used to isolate OSCs. For our studies, viable hOSCs were isolated from ovarian cortex of 6 patients (22-33 years of age), under institutionally approved protocols following written informed consent, by combining cell surface expression of VASA with fluorescence activated cell sorting (FACS). Once hOSCs were established in culture, expression of meiosis-specific markers (Sycp3, Rec8 and Stra8) was assessed. The ability of hOSCs to form oocytes was evaluated by morphology, expression of specific oocyte markers and FACS-based analysis of DNA content for haploid status.
Results: hOSCs maintained in vitro generated large (50 micrometers) spherical cells that expressed several oocyte-specific markers (Nobox, Lhx8, Zp3, Gdf9), with peak numbers of these cells formed within 3 days after splitting the cultures. All three meiotic markers were detected in hOSC cultures in conjunction with oocyte formation, and FACS analysis confirmed the presence of haploid (1n) cells. All three meiotic markers were also detected in ovarian cortical tissue, indicating that the events observed from analysis of hOSCs in culture are also happening in human ovaries in vivo.
Conclusions: our results indicate that isolated hOSCs possess the machinery to initiate and complete meiotic cell division, and generate in vitro what by all criteria tested appear to be oocytes. In addition, cells actively undergoing meiosis are also present in ovaries of reproductive-age women. Collectively, these findings add to the growing body of evidence that oocyte-producing stem cells exist in ovaries of mammals, including humans.
Support: NIH R37-AG012279

T-355

GATA-6 Expression Is Elevated Following TGF-β Induction of a Smooth Muscle Phenotype in Vaginal Progenitor Cells. Marsha K Guess,[1] Jie Xu,[1] Kathleen A Connell,[1] Joshua Johnson,[1] Lloyd G Cantley.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Section of Nephrology, Department of Internal Med, Yale School of Medicine, New Haven, CT, USA.*
Objectives: GATA proteins are a family of zinc finger transcription factors that play an essential role in cardiovascular and visceral endoderm development. GATA-6 is the only GATA protein expressed in vascular smooth muscle and it has been shown to be important in vascular smooth muscle differentiation following vascular injury. We have previously described a novel progenitor cell isolated from human vaginal tissue that we call vaginal adherent stromal cells (VASC) based on their adherent properties in culture. These cells can be induced by TGF-β to differentiate into a smooth muscle phenotype, however, to date, the regulatory factors controlling their differentiation properties is unknown. The goals of this study were to determine if GATA-6 is expressed in VASC and to evaluate the effect of TGF-β on GATA-6 in VASC.
Methods: VASC were isolated from vaginal tissue of women undergoing hysterectomy for benign conditions. Cells were cultured in Dulbecco's Modified Eagle's Medium with 10% FBS. Passage 3 cells were seeded onto a 6-well plate at a density of 50,000 cells/ well and treated with 0, 0.1, 1 or 10ng/ml of TGF-β. On day 7, cells were harvested and protein was isolated. Western blotting was performed with antibodies against GATA-6 as well as differentiated smooth muscle protein markers Calponin and SM22.
Results: At baseline, there was low expression of GATA-6 in VASC. After TGF-β induction, there was a dose-dependent increase in calponin and SM-22. The dose-dependent increases in differentiated smooth muscle markers was associated with a similar dose-dependent increase in GATA-6 protein expression.
Conclusion: GATA-6 is expressed in vaginal progenitor cells and increased when VASC are induced to a smooth muscle phenotype by TGF- β. These findings suggest that GATA-6 may be involved in smooth muscle differentiation of progenitor cells to support the contractility of vaginal tissue.

T-356

Increased Mammalian Target of Rapamycin (mTOR) Corresponds with TGF-β Induced Smooth Muscle Differentiation in Vaginal Adherent Stromal Cells. Marsha K Guess,[1] Jie Xu,[1] Kathleen A Connell,[1] Joshua Johnson,[1] Lloyd Cantley.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Section of Nephrology, Dept of Internal Medicine, Yale School of Medicine, New Haven, CT, USA.*
Objective: Mammalian Target of Rapamycin (mTOR) is a ubiquitously expressed protein kinase that regulates protein synthesis, cycle progression and proliferation in response to nutrient availability. In vascular smooth muscle cells, the mTOR inhibitor Rapamycin (RAP) induces a differentiated smooth muscle phenotype. We have recently described a novel progenitor cell from vaginal tissue that we refer to as vaginal adherent stromal cells (VASC) based on their adherent properties in cell culture. We have successfully differentiated these cells into chondrocytes, osteoblasts, and cells with a smooth muscle phenotype. We are particularly interested in the potential of these cells to produce vaginal smooth muscle and hypothesized that modulation of mTOR would favor vaginal smooth muscle development as shown in vascular smooth muscle differentiation.
Methods: VASC were isolated from vaginal tissue of women undergoing hysterectomy for benign conditions. Cells were cultured in Dulbecco's Modified Eagle's Medium with 10% FBS. Passage 3 cells were seeded onto a 6-well plate at a density of 50,000 cells/ well and treated with 0, 0.1, 1 or 10ng/ml of TGF-β. On day 7, cells were harvested and protein was isolated. Western blotting was performed with antibodies against phosphorylated and total p70S6K (S6K) as well as the differentiated smooth muscle protein markers Calponin and SM22.
Results: In vehicle-treated cells, there was low expression of phosphorylated and total S6K in VASC. After TGF-β treatment, there was a significant dose-dependent increase in calponin and SM-22. The dose-dependent increases in differentiated smooth muscle markers were associated with similar dose-dependent increases in phosphorylated and total S6K protein expression.
Conclusion: Unlike the situation in vascular smooth muscle where mTOR inhibition favors differentiation, vagina-derived VASC treated with TGF-β demonstrate enhanced mTOR activity (S6K phosphorylation), that corresponded with smooth muscle differentiation. These findings suggest that TGF-β acts via the mTOR pahway to control smooth muscle differentiation of progenitor cells to support the contractility of vaginal tissue.

T-357

Mitochondrial Distribution in Human Pluripotent Stem Cells-Derived Cardiomyocytes. Seung-Yup Ku,[1,2] Yoon Young Kim,[1] Yul Huh,[1] Eun-Ju Lee,[3] Sang-Hoon Lee,[3] Seok Hyun Kim,[1,2] Shin Yong Moon,[1,2] Young Min Choi.[1,2] *[1]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul, Korea; [2]Dept. of Ob and Gyn, Seoul National University College of Medicine, Seoul, Korea; [3]Dept. of Ob and Gyn, College of Medicine, Chung-Ang University, Seoul, Korea.*
Functionality of cardiomyocytes (CMs) mainly depends on mitochondria, major cellular organelle of energy metabolism. Differentiation into CMs from human pluripotent stem cells (PSCs) requires energetic activation for the transition of cell state during differentiation. Although the major role of mitochondria in CMs, basic features of mitochondria in human PSCs-derived CMs are unknown. In this study, we tried to demonstrate the dynamic changes of mitochondrial distribution, expression of specific genes and intracellular ROS level in differentiation stage-specific manner from human PSCs.
We induced differentiation into CMs from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) using direct differentiation. We classified human PSCs-derived CMs into early (day 5), middle (day 10) and

late (day 15)-stage, based on the expressions of cardiac-specific and stemness genes. Ultrastructure was evaluated by electron microscope and contents of mitochondria in PSC-derived CMs were evaluated by MitoTracker. Existence of calcium channel and intracellular ROS level was measured using Fluo-4 and DCF-DA, respectively.

The major type of mitochondria in human PSCs-derived CMs was perinuclear mitochondria (PNMs). Mitochondria were abundant in late-stage CMs and increase of content was correlated to the differentiation state. Existence of calcium channel in human PSCs-derived CMs was also analyzed. Intracellular ROS level was also evaluated in human PSCs-derived Cells and the level was lower in human iPSCs-derived CMs.

In conclusion, we demonstrated the dynamic change of mitochondrial distribution in human PSCs-derived CMs. These results clearly showed that the content of mitochondria increased as differentiation proceeded. Furthermore, the biogenesis of mitochondria is critical for differentiation into CMs from PSCs.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (2011-0007944) and Stem Cell Research Center (SC1150), funded by the Ministry of Education, Science and Technology.

T-358

Identification of Novel Genes Implicated in Leiomyoma Formation by Wide Genomic Analysis of Side Population Cells. Aymara* Mas,[1] Irene Cervello,[1] Claudia Gil-Sanchis,[1] Amparo Faus,[1] Jaime Ferro,[1] Antonio Pellicer,[1,2] Carlos Simon.[1,3] *[1]Research, Fundación IVI-IUIVI-INCLIVA Universidad de Valencia, Valencia, Spain; [2]Servicio de Ginecología, Hospital Universitario La Fe, Valencia, Spain; [3]Stem Cell Bank, Unidad Mixta CIPF-UVEG, Valencia, Spain.*

Introduction: Uterine fibroids or leiomyomas represent the most frequent benign tumours being the main cause for hysterectomy in 25-30% of affected women. Despite its high prevalence, their etiology is poorly understood and associated with disorders in somatic stem cell (SSC) population. Side population (SP) technique has been used to isolate SSC in several tissues.

The aim of the present study was to perform genome-wide analysis of putative leiomyoma SSC to identify new biomarkers.

Materials and Methods: Leiomyomas (n=3) were processed after signed informed consent from patients. Tissues were digested and incubated with Hoechst as previously described (Cervelló *et al.,* 2010). Leiomyoma SP (MyoSP) and total fraction (MyoTF) were separated by cell sorter. Isolated cells were used to microarrays analysis using Whole Human Genome Oligo Microarray (Agilent). GenePix Pro 6.0 provided the gene expression profile with non parametric tests. DAVID software allowed to detect differences in biological pathways. Results obtained were validated with PCR.

Results: We identified a total of 100 up- and 53 down-regulated genes differentially expressed in MyoSP *versus* MyoTF. Among the top ten up we identified genes implicated in leiomyomas : *CSF1R* (colony stimulating factor 1 receptor) described in invasive progression in cancers, *ACP5* (acid phosphatase 5) with tumoral features and related with hormones and SP , and *AIF1* (allograft inflammatory factor1) described as a new molecular target in muscle cell proliferation. Concerning the top ten down genes we highlighted: *PLK2* (polo-like kinase2) interacting with hormonal receptors and tumour protein53, TM4SF1 (transmembrane 4 superfamily member) present in tumour cells, and DUSP6 (dual specificity phosphatase 6) described in mesenchymal tumours like Wilms tumor1.

Two up (CCL3, MYL9) and down (TM4SF1, SELE) genes were validated using quantitative PCR.

Conclusions: These findings could provide a set of markers for the identification of human leiomyoma SSC and may contribute to better understanding leiomyoma's physiology.

Funded by SAF 2008-02048, by Fundació Gent per Gent 08/09 and by PROMETEO/2008/163.

*Mas. A and *Cervelló. I contributed equally.

T-359

Inhibition of Ganglioside GD2 Synthesis Suppresses Neural Differentiation of Human Placental Mesenchymal Stem Cells. Jennifer M Ryan, Gregory E Rice, Murray D Mitchell. *UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia.*

Introduction: Placental tissue and its fetal membranes can be considered as one of the most accessible abundant sources of mesenchymal stem cells (MSC). MSC from placental chorion, amnion and villi show high potential for neural differentiation suggesting they might be an ideal source for stem cell neuroregenerative medicine. It is well known that gangliosides which are glycosphingolipids found on all cell membranes play a role in neural development during pregnancy. We, therefore, investigated the effect of silencing ganglioside GD2 during neural differentiation of MSC.

Objective: To investigate whether ganglioside GD2 is involved in neural differentiation of placental-MSC

Methods: MSC were isolated from first trimester placental villi from three different donors. The cells were incubated in 3% O_2 to mimic the physiological oxygen tension of the placental microenvironment *in vivo* and 20% O_2 which is hyperoxic, was used as a comparison group. Neural differentiation was induced by pre-treating the MSC with EGF and FGF for 14 days. Terminal differentiation was induced by culturing MSC on collagen coated plates in neural basal medium supplemented with BDNF and RA for 7 days. In subsequent studies, GD2 was silenced using GD2 specific siRNA. To determine whether GD2 was involved in the differentiation process, the expression of GD2 was analysed in undifferentiated and differentiated MSC. The effect of silencing GD2 was determined by assessing neural lineage marker expression by immunofluorescence in differentiated MSC with and without siRNA.

Results: By day 7, cells cultured in EGF/FGF lost their spindle shaped morphology, decrease in size and became thinner. During terminal differentiation, cells adopted a neural phenotype within 7 days. During neural differentiation, GD2 increased (15%) in differentiated MSC compared to undifferentiated MSC. Furthermore, inhibition of ganglioside GD2 synthesis resulted in MSC suppressed neural differentiation determined by decreased neural marker expression.

Discussion: This is the first report showing that ganglioside GD2 is involved in the regulation of neural differentiation of placental derived MSC. These data demonstrate the importance of ganglioside GD2 in neural differentiation of MSC, providing new insight into the potential clinical application of MSC for neuroregeneration therapy.

T-360

Decreased Sexual Function Scores in Women Post Stem Cell Transplant Compared to Healthy Volunteers. Dana Shanis,[1] Margaret Bevans,[2] Minjung Kwak,[3] Pamela Stratton,[1] Alan DeCherney.[1] *[1]PRAE, Eunice Kennedy Shriver NICHD, NIH, Bethesda, MD, USA; [2]Nursing Department, NIH Clinical Center; [3]Office of Biostatistics Research, NHLBI, NIH.*

Background: Sexual dysfunction is a common long-term issue in female survivors of allogeneic stem cell transplantation (SCT), possibly related to ovarian failure, genital chronic graft-versus-host disease, medication use, depression and self-consciousness.

Objective: To determine if there is a difference in sexual function between women post-transplant and healthy volunteers (HV), and whether post-transplant women using immunosuppression (+immuno) differ in sexual function from those not using these agents (-immuno) in a clinical study of the immunogenicity of Gardasil.

Design: Prospective clinical trial.

Methods: A validated female sexual function questionnaire was completed by women aged 18 to 45 on study entry (Syrjala, 2005). Sexual function overall, interest, desire and arousal scores were compared among groups. Subjects per group (%) who were sexually active and reasons for stopping sexual activity were tabulated. Wilcoxan and Kruskal-Wallis rank sum tests were used.

Results: To date, 22 women (5 – immuno, 7 +immuno, 10 HV) have been studied. 6 (60%) HV reported being sexually active compared to one in each post-transplant group. The overall sexual function score was significantly higher in HV vs all post-transplant (Table 1, $p<0.01$). Interest, desire and arousal scores were significantly higher in HV compared to those post-transplant (Table 1, $p<0.01$). No significant differences were found between women post-transplant on or off immunosuppression in any measure. There were 3 +immuno women who reported stopping sexual activity for lack of sex drive, dyspareunia, bleeding, or inability to orgasm, compared to none in HV or –immuno groups.

Mean sexual function questionnaires subscores

	HV N = 10	PostSCT immuno+ N = 7	PostSCT immuno- N = 5
Overall**	3.30	1.68	1.48
Interest **	3.78	1.41	1.05
Desire **	3.83	1.29	0.60
Arousal **	2.74	0.85	0.45

** p-value <0.01

Conclusions: Women who have undergone SCT have lower sexual function manifested as decreased interest, desire and arousal, and are less likely to be sexually active. Given the importance of sexual function to quality of life in women, screening for sexual dysfunction in the post-transplant population is important.

Support:NCT ID#01092195; ACOG:Hologic 2011-12 Award; Bench to Bedside Award, 2008; PRAE, NICHD; OBS, NHLBI; CC, NIH.

T-361

Localized Uterine Damage Recruits Bone Marrow-Derived Stem Cells Throughout the Endometrium. Hongling Du, Hakan Cakmak, Hugh S Taylor. *Reproductive Endocrinology, OBGYN Department, Yale University, New Haven, CT, USA.*

Objective: Asherman's syndrome occurs most commonly as a result of trauma or infection. We have previously shown that ischemia/reperfusion injury of uterus promoted bone marrow-derived stem cells (BMDSCs) migration to the endometrium, however the role of BMDSCs in Ashermans syndrome has not been characterized. Here a murine model of Asherman's syndrome was created by traumatizing one uterine horn. We evaluated BMDSCs migration, engraftment and transdifferentiation in each horn.

Methods: One wk after myeloablation using busulfan/cyclophosphamide, 10^7 donor BM cells from 8-wk-old C57BL/6 male mice were injected into female recipients of the same age. After 2 wks the mice were randomly divided into two groups. In the injury group , a small incision was made in the right uterine horn at the utero-tubal junction and the horn traumatized using a 24 G needle inserted 2/3 of the way through the lumen, rotated and withdrawn 4 times. The left horn was untouched. In control group, identical surgical incisions were created, however neither horn was damaged. Uteri were collected after 3 months and evaluated by Y chromosome FISH and incubated simultaneously with anti-CD45, anti-F4/80 and anti-cytokeratin. Male testis, spleen and uterine epithelium were used as positive controls. 100,000 cells were counted from each animal.

Results: In the testis positive control approximately 85% of the cells were positive for Y chromosome. We detected an average 1/3000 Y+CD45- cells in controls verses 1/1400 Y+CD45- cells in the damaged right horn and 1/1600 in the undamaged left horn (both P<0.001 compared to control). There was no significant difference between right and left uterine horns.

Table 1. Number of Y+CD45- stem cells recruited/100,000 cells:

Group	Stromal cells	Epithelial cells	Total cells
Control group(n=3)	21	13	33
Damaged right horn(n=3)	55*	14	71
Undamaged left horn(n=3)	50*	14	63

*P<0.001 control vs. either horn for stromal but not epithelial cells; there was no significant difference between the R and L side.

Conclusion: In this study we mimicked the pathophysiology of Asherman's syndrome in a mouse model. Localized damage promoted BMDSCs migration to the endometrial stroma of both uterine horns. Inflammation and injury play an important role in the recruitment BMDSCs to endometrium. Deficient BMSC recruitment may contribute to Asherman's syndrome and modulation of this process offers a potential a novel therapy for this disease.

T-362

Human Amnion Epithelial Cells Modulate Hyperoxia-Induced Neonatal Lung Injury in Mice. Patricia Vosdoganes,[1,2] Rebecca Lim,[1] Eugenia Koulaeva,[1] Siow T Chan,[1] Rutu Y Acharya,[1] Timothy JM Moss,[1,2] Euan M Wallace.[1,2] *[1]The Ritchie Centre, Monash Institute of Medical Research, Clayton, Victoria, Australia; [2]Department of Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia.*

Background: Postnatal exposure to supplemental oxygen increases an infant's risk of developing bronchopulmonary dysplasia among other morbidities. Human amnion epithelial cells (hAECs) can mitigate inflammation and aid tissue repair in fetal and adult lung disease. We hypothesised that hAECs would attenuate hyperoxia-induced changes to neonatal lung structure.

Method: From birth, neonatal mice were exposed to hyperoxia (FiO2 = 85%) for 14 days. Mice were kept in climate-controlled chambers on a 12hour day/night cycle. Human AECs were administered to mice via intraperitoneal injection on days 5, 6 and 7. Controls were exposed to normoxia (FiO2 = 21%) and received IP saline replacement. Body weight was monitored every second day and lungs were collected on day 14 for assessment.

Results: Hyperoxia exposure resulted in reduced neonatal growth, pulmonary inflammation and altered lung structure. Human AECs significantly improved neonatal body weight, and significantly attenuated hyperoxia-induced changes to mean linear intercept, septal crest density, and associated altered gene expression of TGF-β and PDGF-β (all, p<0.05). However, mitigation of hyperoxia-induced changes to alveolar airspace volume, septal tissue volume, tissue-to-airspace ratio, collagen content and leukocyte infiltration was non-significant (all, p≥0.05).

Conclusions: Intraperitoneal administration of hAECs to neonatal mice partially reduced hyperoxia-induced lung inflammation and structural lung damage. These observations suggest that hAECs may be a potential therapy for neonatal lung disease.

F-001

The Sensitivity and Specificity of CT Imaging Compared to Clinical Criteria in the Evaluation of Gynecologic Surgical Complications. Mariam AlHilli, Angelica Garrett, Amy Weaver, Abimbola Famuyide. *Obstetrics and Gynecology, Mayo Clinic, Rochester, MN, USA.*

Objective: To develop clinical diagnostic criteria for the evaluation of patients with complications after gynecologic surgery and determine their predictive value in patients who present with complications after gynecologic surgery in comparison to CT (computed tomography).

Methods: Patients who had abdominal or vaginal gynecologic surgical procedures between 1/1/2008 and 12/31/2009 and underwent CT scan imaging of the abdomen and/or pelvis within 42 days of surgery were identified. Patients who declined research authorization, underwent CT imaging for disease follow up or underwent any of the following: hysteroscopic procedures, wound exploration or examination under anesthesia, were excluded. The proportion of women with abnormal CT scans, and the proportion of women with a subsequent surgical or radiological intervention was determined. The sensitivity and specificity of CT imaging were estimated. Associations between clinical features and the presence of postoperative complications were assessed using Fisher's exact tests.

Results: Overall, 208 scans were performed in 159 patients within 42 days of surgery. There were 94(45.2%) abnormal CT scans. Of all patients, 58 had an intervention related to a postoperative complication (27.9%). Interventions were classified as follows: surgery (n=24), CT or US (ultrasound) guided drain placement or aspiration (n=27), cystoscopic stent placement (n= 2), nephrostomy tube placement (n= 4) and other (n= 2). The sensitivity and specificity of CT imaging in the detection of postoperative complications was 91.9% and 87.7% respectively. When clinical variables were evaluated singly, only an elevated BUN/Creatinine was significantly associated with having any postoperative complication (p=0.02). Combined clinical diagnostic criteria had similar sensitivity and specificity to CT imaging for predicting specific complications including bowel complications (including hernial obstruction), urinary tract complications and pelvic abscess/ hematoma.

Conclusions: CT imaging is highly sensitive in the detection of postoperative gynecologic surgical complications. However, combined clinical diagnostic criteria are similarly predictive of these complications. The use of combined clinical criteria can obviate the use of CT imaging in the postoperative setting thus reducing costs of care and unnecessary exposure to ionizing radiation.

F-002

Deciphering the Roles of Inflammation, Neural Activity, Blood Perfusion and Uterine Contractility in Uterine Pain. Peter Yu,[1] Tamas Jilling,[1] Chaya Segel,[1] Frank F Tu,[1,2] Kevin M Hellman.[1,2] *[1]OB/GYN, NorthShore HealthSystem, Evanston, IL, USA; [2]OB/GYN, University of Chicago, Chicago, IL, USA.*

Objective: It is believed that menstrual pain is caused by high pressure contractions that elicit hypoxia. Recent studies have suggested a role for TRPV1 and TRPA1 in uterine pain due to their increased expression in human chronic pain conditions. Other evidence in humans has supported a role for platelet-activating factor (PAF) as a part of the inflammatory response, because it is elevated the menstrual flow of women with dysmenorrhea. We utilized a mouse model to determine the effects of TRPV1, TRPA1 and PAF receptor activation on uterine contractility and blood perfusion.

Methods: To model uterine pain, a saline filled catheter attached to a pressure transducer was inserted into the uterine horn of lightly anesthetized mice (n=52). After measuring baseline contractility, we examined the effects of either a TRPV1 agonist (capsaicin), a TRPA1 agonist (mustard oil), or a PAFR agonist (CPAF) microinjected or applied externally to a single uterine horn. We then examined how it affected spontaneous contractility, evoked distension induced contractility, local perfusion and autonomic responses. In some animals the effects of a nerve block (1% lidocaine) or a nerve cut (inferior hypogastric) were also examined. ANOVA was statistical evaluation.

Results: Whereas TRPV1 and TRPA1 agonists decreased uterine contractility, PAF increased uterine contractility (peak pressure: 300+/-50%, p <0.05). Nerve cuts after CPAF did not abolish uterine hypercontractility, but nerve cuts before CPAF were capable of eliminating uterine hypercontractility. On the other hand, uterine contractility is not dependent on nerve activity because nerve cuts and nerve blocks do not abolish contractions. Interestingly, both contractions and inflammatory agents appeared to increase perfusion (200+/-30%, p<0.05) suggesting that hypoxic uterine pain is not produced by impaired perfusion. While some damage was observed in the uterus, the most severe inflammation occurred in the bladder (damage score p <0.01), suggesting a neurogenic mechanism responsible for elicited pelvic pain.

Conclusion: These findings demonstrate diverse mechanisms underlie different animal models of uterine pain. Importantly, these findings highlight the importance of neurological pathways in uterine pain as an important early contributing factor to an inflammatory state that bridges organ boundaries.

F-003

Differential Pathways of Hemostasis and Fibrinolysis in Human Endometrial Endothelial Cells. Terry A Jacot, David F Archer. *Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA, USA.*

Menorraghia is the most common type of abnormal uterine bleeding. Many different causes have been suggested, and different treatments are available. Anti-Fibrinolytics are one such treatment targeting the balance between coagulation and fibrinolysis. Fibrin clots can be observed in shedding endometrium during the first 24 hours of menstruation. Endothelial cells maintain this balance through mediators such as von Willebrand Factor (vWF), plasminogen activator inhibitor 1 (PAI-1), and tissue plasminogen activator (tPA). This study investigates the effects of thrombin and Tumor Necrosis Factor-α (TNF-α) on these mediators in human endometrial endothelial cells (HEEC). Modulation by progesterone (P4) was also investigated.

Thrombin (2.5U/ml) or TNF-α (5-10 ng/ml) was added to HEEC cultures for 24 hours. For P4 experiments, 100nM P4 was added for two days prior to thrombin or TNF-α addition. vWF levels in the media were analyzed by western blotting. t-PA and PAI-1 levels in the media were quantitated by ELISA. Data is expressed as percent of control.

Thrombin increased vWF secretion 1.9 times higher compared to control, with a larger effect on tPA and PAI-1 levels at 2.5 and 3.5 times above control, respectively. TNF-α (5 and 10 ng/ml) increased PAI-1 and tPA levels equally between 1.6 and 1.9 times above levels in control cultures, with a marked increase in vWF secretion 2.8 and 3.9 times above control, respectively. All data were statistically significant ($P<0.05$). When P4 was added to HEEC cultures, it did not modulate the TNF-α effects above. While P4 did not change thrombin effects on tPA and vWF, it did reduce the thrombin mediated increase in PAI-1 by 29%.

In summary, progesterone reduces the thrombin-mediated increase of PAI-1 shifting balance toward fibrinolysis. TNF-α mediated effects on PAI-1, tPA, and vWF are progesterone independent, which correlates to the late appearance of TNF-α during the menstrual cycle associated with progesterone withdrawal. Thrombin effects on hemostasis may be more important across the menstrual cycle, while TNF-α may be an important contributory factor to the clots observed during the first 24 hours of menstruation limiting blood loss. Impaired responses to thrombin or TNF-α by endothelial cells should also be considered as a mechanism for menorrhagia.

F-004

Discrepancies of Physician vs Patient Reported Pain during Office-Based Hysteroscopy and Endometrial Suction. Ozgul Muneyyirci-Delale, Asha Chandrareddy, Indra Gjoni, Shawna Tonick, Cassandra Charles, Nanna Osei-Tutu, Jenny Anopa, Dimitre Stefano. *OB/GYN, SUNY Downstate Medical Center, Brooklyn, NY, USA.*

Background: Hysteroscopy is a commonly performed gynecological procedure that is carried out in the operating room under general anesthesia or in the office with or without a topical analgesic spray. If indicated, an endometrial suction can be performed following the hysteroscopy. Although studies show that office-based hysteroscopy and endometrial suction are tolerated by most patients, pain is often a concern for the patient when considering to undergo these procedures. We assessed discrepancies between physicians and patients in reporting of pain during these office-based procedures.

Material & **Methods**: This study received IRB approval. 150 women consented to undergo office-based hysteroscopy and/or endometrial suction at Downstate Medical Center. During the procedures, pain scores were obtained at 3 points (during the tenaculum application [PT], hysteroscopy [PH] and endometrial suction [PS]) via 2 methods. First, using the Revised Faces Pain Scale at each of the 3 points, the physician recorded the intensity of their patient's facial expression. Directly following this, at each of the 3 points, the patient verbally reported their own level of pain on a numerical scale 0-10 with 10 representing the worst pain imaginable. Patients also completed a questionnaire about their gynecological history and their charts were reviewed for demographic information. Data was analyzed using Pearson's Correlations and student's t-test.

Results: Pain reported on both scales at all 3 points during the procedure were strongly correlated ($p< 0.0001$ for all). However, physician-reported scores were significantly lower ($p= 0.03$) than patients. PT and PS were moderately correlated on both scales ($p< 0.0001$ for both), while PH and PS were moderately correlated only with the physician's scale ($p= 0.001$).

Conclusion: Patient reported and physician observed pain scales are both viable means of assessing pain during office-based hysteroscopy. Additionally, PT could be used to predict the level of pain to be encountered during the endometrial suction. In the event the patient experiences severe pain, the procedure under conscious sedation or general anesthesia should be strongly reconsidered.

F-005

NADPH Oxidase p22-Phox Gene Polymorphism in Women Is Associated with the Development of Postoperative Adhesions. Ghassan M Saed, Nicole M Fletcher, Awoniyi Awonuga, Mohammed G Saed, Zhongliang Jiang, Husam M Abu-Soud, Michael P Diamond. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*

Introduction: Hypoxia, as a result of tissue injury, is a key element in the pathogenesis of postoperative adhesions, which contributes to significant morbidity in women and men. Hypoxia irreversibly induces normal peritoneal fibroblasts to acquire the adhesion phenotype. NADPH oxidase is an important source of superoxide in human cells. Previous studies have suggested an association between polymorphisms in the NADPH oxidase gene and the persistent activity of this enzyme in various conditions.

Objective: The objective of this study was to determine whether adhesion fibroblasts manifest a higher degree of the C242T NADPH polymorphism than normal peritoneal fibroblasts, and whether this polymorphism is inducible by hypoxia.

Methods: The expression of NADPH oxidase in normal human peritoneal and adhesion tissues and fibroblasts was determined utilizing immunohistochemistry, Western blot and real-time RT-PCR. The NADPH oxidase polymorphism in exon 4, at position 242, which leads to the non-conservative substitution of histidine-72 with a tyrosine was analyzed using specific TaqMan probes that quantitate the abundance of this polymorphism in normal peritoneal and adhesion fibroblast cell cultures before and after exposure to hypoxia (2% O_2, 24 hrs). A Student's t-test was used and values of $p < 0.05$ were considered statistically significant.

Results: NADPH oxidase was overexpressed in adhesion tissues and fibroblasts as compared to normal peritoneal tissues and fibroblasts. Additionally, adhesion fibroblasts exhibited an increase the NADPH oxidase polymorphism (53.7%, $p<0.05$) as compared to normal peritoneal fibroblasts (25.2%, $p<0.05$). Moreover, exposure of normal peritoneal fibroblasts to hypoxia significantly increased the acquisition of this polymorphism, from 25.2% to 51.8% ($p<0.05$). Adhesion fibroblasts had no further increase in the C242T polymorphism following exposure to hypoxia.

Conclusion: An association between NADPH oxidase polymorphism and the development of the adhesion phenotype may identify the cause of this "irreversible" modification of the adhesion phenotype and will provide the opportunity for limiting its acquisition and/or altering its persistence.

F-006

Phase I Combination Gefitinib and Methotrexate to Medically Treat Ectopic Pregnancy. Monika Skubisz,[1] Andrew Horne,[3] Ann Doust,[3] Euan Wallace,[1] Colin Duncan,[3] Hilary Critchley,[3] Stephen Tong.[2] *[1]Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia; [2]Obstetrics & Gynaecology, University of Melbourne, Heidelberg, Victoria, Australia; [3]Obstetrics & Gynaecology, University of Edinburgh, Edinburgh, Scotland, United Kingdom.*

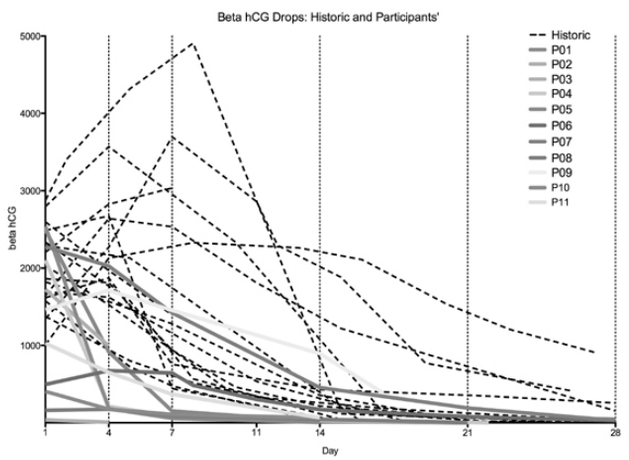
Background

1-2% of all pregnancies are ectopic. We have performed pre-clinical studies showing methotrexate and gefitinib (Epidermal Growth Factor Receptor [EGFR] Inhibitor, orally bioavailable) are supra-additive in regressing placental tissue. Here we progressed this concept to the clinic, treating humans with ectopic pregnancies.

Objective

To perform a phase I study to see whether gefitinib and methotrexate is safe, well-tolerated and effective in treating ectopic pregnancies.

Methods
We set out to recruit 12 women with haemodynamically stable ectopic pregnancies from Edinburgh and Melbourne. We administered methotrexte 50mg/m2 intramuscularly on day 1 plus 250mg of oral daily gefitinib in a dose-escalation protocol (1 tablet in first 3 women; 3 tablets next 3; 7 tablets final 6 women). We monitored for toxicity and side effects by history, examination and biochemical tests. To obtain early efficacy data, we graphed rate of decline in serum ßhCG against a contemporaneous cohort given single dose methotrexate.
Results
Phase I is almost completed with 11 of 12 women recruited. The combination is well tolerated with no significant toxicities noted. All women resumed menses. Among some participants, we observed precipitous early declines in serum ßhCG with prompt resolution of their ectopic pregnancies (Figure 1). One woman successfully treated of an ectopic pregnancy in her only remaining fallopian tube subsequently conceived a spontaneous intrauterine pregnancy. We will be progressing to a phase II study.
Conclusion
Adding gefitinib tablets to methotrexate is well tolerated and efficacious in medically treating ectopic pregnancies of any size.
Figure 1. Decline of Serum ßhCG Over Time for Participants (combination gefitinib and methotrexate treatment with day 1 ßhCG <3000IU/L (solid lines)) vs. Historic Cohort (single agent methotrexate where day 1ßhCG is 1000-3000IU/L (black, dotted lines)).



F-007

How To Reduce Caesarean Section Rate Improving Neonatal Outcomes. C Mastromatteo,[1] SF Deiana,[2] E Giambattista,[1] X Santopietro,[1] PM Villa,[1] P Alimondi,[3] A Meloni,[2] A Antonelli,[2] GB Melis,[2] DE Rinaldo,[4] FA Ragusa.[1] *[1]Ob/Gyn Dept, I.C.P. Sesto San Giovanni; [2]Ob/Gyn Dept, Cagliari; [3]Ob/Gyn Dept, Palermo; [4]Ob/Gyn Dept, Seriate.*
AIM OF THE STUDY
The objective of our study was to apply risk management techniques in order to reduce CS rate without increasing maternal and neonatal morbility.
MATERIALS AND METHODS
Retrospective study comparing CS performed in two six-months periods (500 deliveries each) in Sesto S.Giovanni Obstetrical Unit. The new Director of the Obstetrical Unit improved the clinical/assistential performance, introducing:
-daily audit and discussion of clinical cases
-revision of clinical protocols
-intrapartum ultrasound
-attention to the psychological well-being of women in labour
-new classification of CTG traces in the second stage of labour.
We used Robson's classification to compare results before and after the above mentioned intervention.
Each case of CS was classified according to Robson's criteria into one of ten mutually exclusive categories according to obstetric characteristics. We performed a student T-test altogether and detailed for every single class to evaluate the differences in CS proportions.
RESULTS
CS rate was 26% (130/500 del.) in pre-intervention period (Group 1) and 20% (99/500 del.) in post intervention period (Group 2) (p-value 0,0242).
Overall CS rate was reduced by approximately one forth.
The reduction was observed mainly in Robson class 1,2 and 4.
The considerable reduction in CS rate corresponded to a statistically significant reduction of newborns with 5-minute Apgar score < 7 and umbilical cord arterial pH ≤ 7.00 (2,8% in group 1 vs 0,8% in group 2) (p-value 0,0174).
CONCLUSIONS
In the present study, we found a significant change in the pattern of CS rates that could be attributable to the implementation of continuous Audit and Feedback. Our study showed a significant reduction in CS rate especially in nulliparous women with uneventful term pregnancy, a large contributor to the overall CS rate increase, by a reduction of the more subjective indications to CS (non reassuring fetal status and arrest of dilation).
With continuous clinical meetings a leader can modify the incongruous clinical behaviours.
Continuous Audit and Feedback together with a better obstetric care reflects on a significant decrease in CS rate without increasing neonatal morbidity.

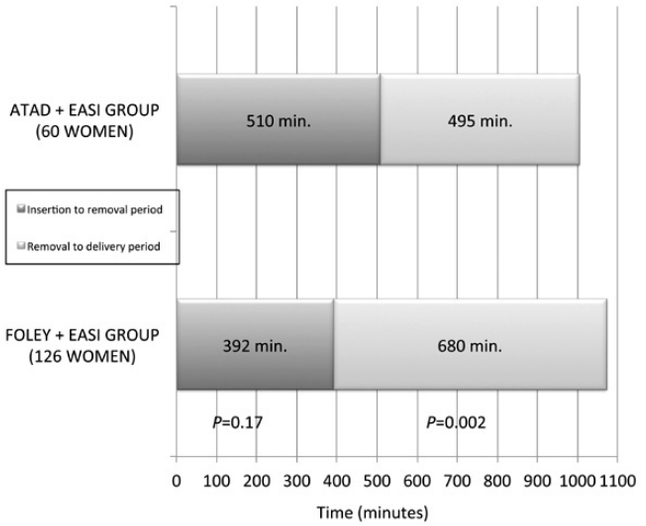
F-008

Cervical Ripening with Extra Amniotic Saline Infusion: A Randomized Comparison of Two Mechanical Devices. Elad Mei-Dan,[1] Asnat Walfisch,[1] Constanza Valencia,[2] Mordechai Hallak.[1] *[1]Obstetrics & Gynecology, Hillel Yaffe Medical Center, Hadera, Israel; [2]Faculty of Medicine, Technion, Isreal Institute of Technology, Haifa, Israel.*
OBJECTIVE:
To compare the efficacy of two mechanical devices for cervical ripening: Foley Catheter and the newly introduced Cook Cervical Ripening Balloon, both with extra-amniotic saline infusion (EASI).
STUDY DESIGN:
Women at term with a singleton pregnancy who presented for labor induction were randomly assigned to the Foley catheter+EASI or the Cook Cervical Ripening Balloon+EASI. Outcome measures included ripening success, time from device insertion to delivery, cesarean section rates, maternal and neonatal adverse outcome.
RESULTS:
The study was completed by 186 women. Ripening success was comparable between the groups (Table). Time from balloon insertion to delivery was significantly shorter, and cesarean section rate was significantly lower in the Cook+EASI group, when compared to the Foley+EASI group (Figure+Table). There were no significant differences in maternal characteristics, satisfaction and other outcomes.
CONCLUSIONS:
This study is the first documented assessment of the newly introduced Cook Cervical Ripening Balloon with EASI method. Our results suggest this method to be superior and faster for initiating a labor process, resulting in a successful vaginal delivery, compared to the Foley catheter with EASI method.

Maternal Characteristics and outcomes

Maternal demographics	Foley + EASI Group (n=126)	Cook + EASI Group (n=60)	P value
Maternal Age (years)	29.2±5.3	29.2±5.2	0.98
BMI (Kg/m2)	29.8±5.5	27.9±4.7	0.22
Obstetrical characteristics			
Parity (%primiparity)	50.8	48.3	0.57
Gestational age (weeks)	39.3±1.8	39.2±1.7	0.76
Outcomes			
Ripening success (%)	92.7	96.4	0.55
Balloon insertion to delivery time (median, hours:min)	15:50	14:19	0.04
Cesarean section (%)	20	8.3	0.05
Birth-weight (gr)	3113±537	3129±505	0.84
Total hospitalization (days)	5.6±2.9	4.2±1.8	<0.001

Friday



F-009

VBAC Prediction Model for Use at the Time of Admission. Torri D Metz,[1] Gregory J Stoddard,[2] Erick Henry,[3] Marc Jackson,[3] Calla Holmgren,[3] Sean Esplin.[3] *[1]Obstetrics and Gynecology, University of Utah; [2]Study Design and Biostatistics Center, University of Utah; [3]Obstetrics and Gynecology, Intermountain Medical Center.*

Objective: Our goal was to create a simple tool for predicting the chance of successful trial of labor after cesarean (TOLAC) in the pregnancy following a primary cesarean (C/S) with variables available at the time of admission to Labor and Delivery.

Methods: Electronic medical record data for all deliveries at 14 regional hospitals over an 8-year period were reviewed. Women with one C/S and one subsequent delivery in the data set were included. A prediction model for successful VBAC was created using logistic regression, then validated with bootstrapping.

Results: 5,445 women had a primary C/S and a subsequent delivery. 1,169 (21.5%) of these women underwent TOLAC with their next pregnancy. The rate of successful TOLAC among these women was 80%. Multivariate logistic regression identified five variables that were associated with successful TOLAC: age<35 yrs (OR 1.9, 95% CI 1.1,3.4), history of vaginal birth (OR 2.8, 95% CI 1.9,4.1), BMI<30 (OR 1.6, 95% CI 1.1,2.4), each point of Bishop score (OR 1.3, 95% CI 1.2,1.4), absence of recurrent indication (OR 2.0, 95% CI 1.3,3.1). The area under the ROC curve was 0.71. These variables were then integrated into a prediction model based on the initial cervical exam to calculate an integer VBAC score (Figure).

Figure. VBAC Score Calculator

Calculate Bishop Score based on cervical exam at time of admission

Add 4 points for history of vaginal delivery

Add 2 points if BMI≤30

Add 3 points if primary cesarean was not for a recurring indication

Add 2 points if maternal age is < 35 years at time of admission

Total VBAC Score =

The VBAC score was correlated with the TOLAC success rate (Table).

Table. Chance of Successful VBAC Based on Calculated VBAC Score

Calculated VBAC Score	% Chance of Successful VBAC (95% CI)
4	18.6 (6.4, 20.7)
5	23.2 (8.6, 24.4)
6	28.5 (11.9, 29.2)
7	34.3 (16.8, 35.2)
8	40.4 (23.4, 42.0)
9	46.7 (31.9, 49.3)
10	52.9 (46.7, 56.7)
11	59.0 (51.8, 63.7)
12	64.8 (61.1, 70.0)
13	70.2 (68.8, 75.5)
14	75.1 (74.8, 80.1)
15	79.5 (79.2, 83.9)
16	83.3 (82.4, 86.8)
17	86.5 (84.7, 89.0)
18	89.2 (86.4, 90.6)
19	91.4 (87.6, 91.8)
20	93.2 (88.5, 92.6)
21	94.6 (89.2, 93.2)
22	95.7 (89.7, 93.7)
23	96.7 (90.1, 94.1)

Conclusion: Prediction of TOLAC success at the time of admission is highly dependent on the initial cervical exam. Patients with an admission score <9 have a likelihood of TOLAC success of 50% or less. Patients with an admission score of >16 have a TOLAC success rate greater than 85%. This simple VBAC score can be utilized in counseling women who are considering a TOLAC.

F-010

How Do Good Candidates for TOLAC Who Choose Elective Repeat Cesarean Differ from Those Who Choose TOLAC? Torri D Metz,[1] Gregory J Stoddard,[2] Erick Henry,[3] Marc Jackson,[3] Calla Holmgren,[3] Sean Esplin.[3] *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA; [2]Study Design and Biostatistics Center, University of Utah; [3]Obstetrics and Gynecology, Intermountain Medical Center.*

Objective: Many women who are good candidates for a trial of labor after cesarean (TOLAC) choose to have an elective repeat cesarean (ERC) for their next delivery. Our aim was to compare good TOLAC candidates who chose ERC to those who chose TOLAC.

Methods: Electronic medical record data for all deliveries at 14 regional hospitals over an 8-year period were reviewed. Women with a primary C/S and one subsequent delivery in the data set were included. The choice of ERC versus TOLAC was assessed in the first delivery following the primary C/S. Women with ≥ 70% chance of successful VBAC as calculated at the first prenatal visit using the regression equation by Grobman et al (2007) were considered good candidates for TOLAC. Good candidates who chose an ERC were compared to those who chose a TOLAC.

Results: 5,445 women had a primary C/S and a subsequent delivery. 3,624 (66.6%) of these women were calculated to be good TOLAC candidates at the next delivery. Of this group, 2,698 (74.4%) chose ERC, and 926 (25.6%) chose TOLAC. The women choosing an ERC were less likely to be uninsured, and more likely to be unmarried, obese, and managed by a general Ob-Gyn (Table). Women with a prior vaginal delivery and those cared for by a certified nurse midwife were more likely to choose a TOLAC (Table).

Table. Characteristics of Women Who Are "Good Candidates" for TOLAC

Demographic Variable	Chose Elective Repeat Cesarean (n=2,698)	Chose Trial of Labor After Cesarean (n=926)	P value
Race, n(%)			
White	2,527 (93.7)	870 (94.0)	0.75
Hispanic	86 (3.2)	24 (2.6)	0.75
African American	6 (0.2)	3 (0.3)	0.75
Other	79 (2.9)	29 (3.1)	0.75
Insurance Type, n(%)			
Private	2,070 (76.7)	720 (77.8)	0.52
Medicaid/Medicare	588 (21.8)	180 (19.4)	0.20
Uninsured	40 (1.5)	26 (2.8)	0.03
Maternal Age, yrs±SD	27.4±4.3	27.7±4.4	0.09
Marital Status, n(%)			
Married	2452 (90.9)	868 (93.7)	0.01
Primary Cesarean for Recurring Indication, n(%)	100 (3.7)	26 (2.8)	0.20
History of Vaginal Delivery, n(%)	681 (25.2)	354 (38.2)	<0.001
Body Mass Index > 30, n(%)	343 (12.7)	74 (8.0)	<0.001
Delivery at a Tertiary Hospital, n(%)	1,218 (45.1)	388 (41.9)	0.09
Managing Provider, n(%)			
General Ob-Gyn	1399 (51.9)	438 (47.3)	0.03
MFM	36 (1.3)	8 (0.9)	0.26
Certified Nurse Midwife	25 (0.9)	30 (3.2)	<0.001
Family Practice	138 (5.1)	35 (3.8)	0.13

Conclusion: Nearly three-quarters of the good candidates for TOLAC chose ERC. Differing demographic characteristics and provider counseling may play a role in whether women who are good candidates choose a TOLAC.

F-011

Successful Management of Deliveries of a Woman with PAI-1 Deficiency – A Critical Role of PAI-1 in the Maintenance of Pregnancy. Kotomi Nagahashi,[1] Takayuki Iwaki,[2] Keiko Muramatsu,[1] Kaori Yamazaki,[1] Naoaki Tamura,[1] Hiroaki Itoh,[1] Naohiro Kanayama.[1] *[1]Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka-ken, Japan; [2]Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka-ken, Japan.*

Plasminogen activator inhibitor-1 (PAI-1) inactivates tissue-type or urokinase-type plasminogen activators. There have been several reports of PAI-1 deficiency, who usually suffered a tendency for massive bleeding. We managed two deliveries of a woman with bleeding tendency of unknown etiology, who was later diagnosed as PAI-1 deficiency. She has multiple episodes of bleeding, i.e. omphalorrhagia at birth, postoperative bleeding after an operation for a ventricular septum defect patch at 5-year-old. At 16-year-old, she experienced a massive hypermenorrhea (total 6L), and her uterine arteries were ligated. Her first pregnancy terminated in a spontaneous miscarriage with massive genital bleeding at 19 weeks' gestation. In her second and third pregnancies fresh frozen plasma (FFP) was administrated throughout the course of pregnancy (total 361 and 378 units, respectively) because of an unidentified coagulation factor deficiency. In her second pregnancy, genital bleeding continued from 11 weeks of gestation and gradually increased. An emergency cesarean section was performed due to uncontrollable massive genital bleeding with premature uterine contractions at 32 weeks of gestation and an immature male baby weighing 1736 g was born. In her third pregnancy, an emergency cesarean section was performed due to similar uncontrollable massive genital bleeding with premature uterine contractions at 27 weeks of gestation and an immature female baby weighing 978 g was born. At 47-year-old, she was re-evaluated. Euglobulin clot lysis time was remarkably shortened, and her PAI-1 antigen levels were undetectable, indicating that PAI-1 was completely absent. DNA sequencing of her SERPINE1 gene showed a mutation; 1-bp duplication(C) at exon 3. This mutation caused the frame shift and resultantly the mutated protein of PAI-1was barely expressed. It was plausible that PAI-1 in the administrated FFP contributed to the maintenance of her second and third pregnancies. As far as we know, this is the first report of the successful management of pregnancies and deliveries of a woman with PAI-1 deficiency, highlighting a critical role of PAI-1 in the maintenance of pregnancy.

F-012

SGA Preterm Neonates Born to Mothers without Intrauterine Inflammation Are at Risk for the Fetal Inflammatory Response Syndrome and Have a Lower Risk of RDS. Chan-Wook Park, Bo Hyun Yoon, Joong Shin Park, Jo Kwan Jun. *Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Korea.*

OBJECTIVE: The fetal inflammatory response syndrome (FIRS) was originally described in patients with preterm labor and intact membranes (PTL) and preterm premature rupture of membranes (preterm PROM). However, inflammation is a non-specific mechanism of host defense which may be triggered by non-infection related "danger signals". Small for gestational age (SGA) neonates are at risk for adverse outcomes. The purpose of this study was to determine if SGA preterm neonates born to mothers without intrauterine inflammation are at an increased risk for FIRS and neonatal respiratory distress syndrome (RDS).

METHODS: The relationship among SGA, FIRS and neonatal RDS was examined in 92 singleton preterm births (24.5wks≤gestational age(GA)<33.5wks) within 3 days of amniocentesis born to mothers without intrauterine inflammation. Intrauterine inflammation was considered to be present if there was intra-amniotic infection/inflammation or histologic chorioamnionitis. Intra-amniotic inflammation (IAI) was defined as an elevated amniotic fluid (AF) matrix metalloproteinase-8 (≥23ng/ml), and FIRS as a high C-reactive protein(≥200ng/ml) in umbilical cord plasma at birth. SGA was defined as a birth weight below the 5th percentile for GA.

RESULTS: 1) SGA was present in 32% of this population; 2) Preterm SGA neonates had a higher rate of FIRS and umbilical arterial (UA) pH≤ 7.15 than non-SGA neonates (FIRS, 31% vs 8%, p<.05; UA pH≤7.15, 33% vs 13%, p=.05) after adjusting for the cause of preterm birth; 3) However, SGA neonates had a lower rate of RDS than non-SGA neonates (24% vs 56%; p<.05).

CONCLUSION: SGA preterm neonates born to mothers without intrauterine inflammation are at risk for FIRS but have a lower rate of RDS.

F-013

Preterm Birth and Antenatal Corticosteroid Administration: How Good Are We at Achieving Optimal Timing? Ashlie Tronnes, Amanda Kinhnarath, Aaron B Caughey, Janice Snyder, Leonardo Pereira. *OBGYN, Oregon Health & Science University, Portland, OR, USA.*

OBJECTIVE: Antenatal corticosteroids (ACS) are most effective at improving neonatal outcomes when a full course (2 doses, 48 hours prior to delivery) is administered prior to 34 weeks gestation (wk) and within 7-14 days of preterm birth (PTB). However, in many pregnancies the timing of ACS falls outside this optimal treatment window, reducing neonatal benefits. Our objective was to determine the proportion of patients receiving ACS in an optimal treatment window.

STUDY DESIGN: Prospective cohort study of patients at OHSU with threatened PTB and singleton pregnancies enrolled from 2007-2011. Three groups of patients were designated based on interval from presentation to delivery. In group 1, delivery occurred < 48 hours, in group 2, 2-14 days, and in group 3, > 14 days after presentation. Analysis of variance was used to compare cervical dilation across groups. Statistical significance was defined as $p < 0.05$.

RESULTS: From a total of 80 patients, 1 was lost to follow up, leaving 79 in the final cohort. Group 1 consisted of 8 patients (10%), group 2 consisted of 19 patients (24%), with the remaining 52 in group 3 (66%). Maternal age, parity, cervical dilation, and prior PTB did not differ across groups. Within group 2, 15/19 patients delivered within 2-7 days from ACS administration (19% of total cohort). Delivery occurred < 34 wk in 42%, between 34-37 wk in 21%, and after 37 wk in 37% of the total cohort. Among the 33 patients who delivered < 34 wk, 8 were in group 1, 16 in group 2 (13/16 within 2-7 days), and 9 in group 3.

CONCLUSION: Our ability to deliver ACS in an optimal treatment window to improve neonatal outcomes is poor. In a prospective trial of women with singleton pregnancies and threatened PTB, only 24% received ACS in a window of 2-14 days from presentation to delivery; and only 19% in a window of 2-7 days. Less than half (48%) of patients with delivery < 34 wk received ACS within 2-14 days of delivery, and 37% of patients who received ACS delivered at term (> 37 wk). Improved prediction of PTB timing would improve neonatal outcomes by improving ACS timing.

F-014

A Prospective Profile of the Maternal Plasma Proteome during Early Pregnancy. Gregory E Rice,[1] Sebastian Illanes Lopez,[2] Alejandra Perez-Sepulveda,[2] Hsiu-Wen Chan,[1] Kanchan Vaswani,[1] Murray D Mitchell.[1] *[1]Centre for Clinical Research, University of Queensland, Herston, QLD, Australia; [2]Departamento De Ginecologia Y Obstetricia, Universidad De Los Andes, Las Condes, Santiago, Chile.*

Introduction The development of screening tests to identify pre-symptomatic women who subsequently develop complications of pregnancy is a prerequisite to implementing efficacious treatment. Selection criteria for biomarkers of complications of pregnancy include: expression is independent of gestational age during the sampling period; and the biomarker is present in all subjects of a specific category. There is a paucity of data that define changes in maternal plasma protein expression during normal early pregnancy. The aim of this study was to define early pregnancy changes in plasma proteins in women who subsequently experienced normal pregnancies. The null hypothesis to be tested was that the expression of maternal plasma protein (as displayed by 2-dimensional gel electrophoresis, 2DE) does not change during 6-12 weeks of pregnancy.

Methods: The study was a prospective time series design in which plasma samples were collected weekly from 6-12 weeks of gestation from 4 women. High abundance plasma proteins were depleted prior to analysis. Proteins (50ug) were labeled with fluorescent Cy2 or Cy3 dyes and then displayed using 2DE (13 cm non-linear, pH 3-10). Protein expression was defined by average normalized spot volume. Gestational variation of proteins that were represented in all gels (i.e. common proteins) was assessed by one-class, time-series analysis.

Results: Total plasma protein concentration was not significantly affected by gestational age ($p = 0.64$). Eighty-eight protein spots were identified as common to all gels. Sixty of these were identified by MALDI Tof/Tof MS. At a false discovery rate of 1%, one-class, time-series analysis identified 6 proteins as significantly associated with gestational age: 4 increased and 2 decreased over the 7-week period.

Conclusions: The expression of a subset of common maternal plasma proteins (6 of 88), displayed by 2DE, changes significantly during 6-12 weeks of pregnancy. The majority of 2DE-display plasma proteins, however, remain stable over this period. With respect to developing early pregnancy screening tests for assigning risk of subsequent complications of pregnancy, these data highlight the requirement to establish how the expression of putative biomarkers changes during the proposed sampling period.

F-015

Difficult Adaptation to Birth and Neonatal Encephalopathy. DE Rinaldo,[1] FA Ragusa,[2] A Del Prete,[3] L Danti,[4] MR Di Tommaso,[5] S Felis,[6] L Trespidi,[7] A Locatelli,[8] S Alberico.[9] *[1]Ob/Gyn Dept, Seriate; [2]Ob/Gyn Dept, Sesto San Giovanni; [3]Neon Dept, Lecco; [4]Ob/Gyn Dept, Brescia; [5]Ob/Gyn Dept, Firenze; [6]Ob/Gyn Dept, Genova; [7]Ob/Gyn Dept, Mangiagalli-MI; [8]Ob/Gyn Dept, Monza; [9]Ob/Gyn Dept, Trieste.*

Prospective observational multicentric study performed in different Obstetric Units in the North of Italy.

One of the aims of the study was to understand the proportion of babies born with DAB that are going to develop a degree 1-2 or 3 neonatal encephalopathy (NE).

We excluded pregnancies complicated by IUGR, congenital anomalies, antenatal infections and multiple pregnancies.

We recruited singleton uneventful term pregnancies (>37 wks g.a.) with one or more of the following risk factors:

1) 5-minute Apgar score < 7
2) Umbilical cord arterial pH ≤ 7.00 and/or B.E. ≥ -12
3) Need for intubation or resuscitation at birth.

We defined "difficult adaptation to birth" (DAB) the presence of one or more of these factors.

Between 2005 and 2009 129 newborns were enrolled, 86 cases coming just from 2 Units, that were recruiting babies sistematically. The incidence of DAB recorded in is these two Depts was 0,56%.

In 47 cases babies were delivered by a spontaneous vaginal delivery, 39 by instrumental vaginal delivery (vacuum), 40 by emergency CS and 3 by elective CS.

- 13 newborns on the 129 enrolled developed NE (1,01%)
- 81 on 129 had arterial pH ≤ 7.00: 7 of them (9%) developed NE.
- 35 on 129 had 5-minute Apgar score < 7: 9 of them (26%) developed NE.
- 20 on 129 needed intubation or resuscitation at birth: 10 of them (50%) developed NE.

Since on the 13 newborns developing NE, 10 required intubation or resuscitation we decided to analyze the factors pH and Apgar score within this group of 10 babies.

On the10 babies intubated who subsequently developed NE, 6 (60%) had poor umbilical cord gases as well, 9 (90%) had also a low 5-minute Apgar score.

From our data we can therefore conclude that in singleton uneventful term pregnancies that end up with a baby with a difficult adaptation to birth, the need for intubation or resuscitation represents the most unfavourable prognostic index; the prognosis is even more adverse if 5-minute Apgar score is < 7.

Our study shows that the major component to the development of NE is the incapacity to breathe spontaneously at birth, that is often determined by other causes than acidosis.

The strength of our study is that is the only prospective one published.

The limit is the small sample size.

F-016

Pregnancy during Peritoneal Dialysis in a Patient with End-Stage Renal Disease Due to Vasculitis Treated with Cyclophosphamide. Rita Mocciaro, Angela Sacchinelli, Roberta Venturella, Michele Morelli, Zullo Fulvio. *Gynecology and Obstetrics, University "Magna Graecia", Catanzaro.*

INTRODUCTION: Pregnancy in dialysis patients with the end stage renal disease (ESRD) is uncommon. In particular, the rate of pregnancy in peritoneal dialysis (PD) patients is lower than in haemodialysis (HD) patients.

This has been ascribed to several factors such as the large volume of fluid in the intraperitoneal space that can interfere with transport of the ovum from the ovary to the fallopian tubes, the presence in this fluid of hypertonic dextrose that can damage the ovum and the possibility that recurrent peritonitis can result in tubal obstruction.

In half of cases, ESRD was associated with ANCA small-vessel vasculitis. Fertility in patients with vasculitis is reduced following treatment with cyclophophamide. This cytotoxic drug could cause damage ovarian pregranulosa cells and cortical blood vessels, and oocyte and follicle loss associated with apoptosis.

It is commonly known that in these pregnancies, the prognosis for the mother and the fetus is poor.

CASE PRESENTATION: In this report, we present the case of a young woman subject to a continuous ambulatory peritoneal dyalisis (CAPD) for ESRD caused by acute systemic vasculitis treated with cyclophosphamide that became pregnant.

This patient delivered a healthy infant, albeit premature; with a normal physical and intellectual development after 12 months from delivery.

CONCLUSION: The peculiarity of this case is that the patient achieved a pregnancy notwithstanding treatment with cyclophosphamide and peritoneal dialysis. Moreover, she has maintained a pregnancy without complications and delivered a healthy newborn.

F-017

Defibulation in Labor. Abdulrahim A Rouzi. *Obstetrics and Gynecology, King Abdulaziz Uinversity.*

Objective: To report a primigravida with Type III Female Genital Cutting/ Mutilation (FGC/M) who came in labor without previous registration and delivered vaginally with intrapartum defibulation.

Materials and Methods: A 21 years-old Sudanese primigravida at term was seen in the emergency room of King Abdulaziz Uinversity Hospital at 2 am in labor. She was not seen in the antenatal clinics before. She did not seek any medical advice during pregnancy. On examination, she had Type III Female Genital Cutting/Mutilation (FGC/M). The cervix was 4 cm dilated and the head was at zero station. The situation was discussed with the couple and decision was made to follow the hospital policy of doing intrapartum defibulation.

Results: She progressed normally in labor and had spontaneous vaginal delivery with defibulation with crowning of the head.

Estimated blood loss was 300 cc. She was sent home with her baby in good general condition on the second post partum day.

Conclusions: In the West, lack of experience in managing FGC/M in labor led to unnecessary cesarean delivery or vaginal delivery with midline episiotomy and spontaneous separation of the infibulation scar with extensive vaginal lacerations. This is no longer an acceptable practice. The American College of Obstetricians and Gynecologists (ACOG) guidelines recommends doing defibulation during the second trimester under spinal anesthesia. The British Royal College of Obstetricians and Gynaecologists guidelines recommends doing defibulation either before or during pregnancy in specialized clinics by "a senior person with extensive experience in dealing with reversal of the

mutilation" in order to perform an adequate vaginal examination and to prevent perineal dystocia. With appropriate care, anxiety is completely allayed. With proper training, all health care providers should be able to perform antepartum and intrapartum defibulation.

F-018

Labor Dystocia – Risk of Recurence and Mode of Delivery in Second Labor. Anna Sandstrom,[1,2] Sven Cnattingius,[1] Anna-Karin Wickstrom,[1] Olof Stephansson.[1,2] *[1]Department of Medicine Solna, Clinical Epidemiology Unit, Karolinska University Hospital, and Institutet, Stockholm, Sweden; [2]Department of Women's and Children's Health, Division of Obstetrics and Gynaecology, Karolinska University Hospital, and Institutet, Stockholm, Sweden; [3]Sweden.*

OBJECTIVE: To investigate risk of recurrence of labor dystocia and mode of delivery in second labor and whether this is influenced by first labor, infant and maternal characteristics.

STUDY DESIGN: A population-based cohort study using the Swedish Medical Birth Register between 1992 and 2006. The study included 231,936 women who gave birth to their first and second live singleton infants, born in cephalic presentation at 37 gestational weeks or later with spontaneous onset of both labors. We used logistic regression analysis to estimate crude and adjusted odds ratios (OR), with associated 95% confidence intervals (CI), for dystocia and mode of delivery in second labor, adjusted for first labor, infant and maternal characteristics.

RESULTS: Among women with labor dystocia and vaginal delivery in first labor 10.3 % had recurrence of dystocia, a four-fold increased risk of dystocia in second delivery (crude OR 4.02; 95% CI 3.66-4.00). If previous dystocia and trial of labor after cesarean (TOLAC) 34.1 % had recurrence of dystocia. Long interpregnancy interval, maternal age ≥ 35, maternal overweight, short maternal stature, not co-habiting with the baby´s father and post term pregnancy were associated with an increased risk of dystocia in second labor. If TOLAC 32.1 % of women with and 22.9% of women without previous labor dystocia had cesarean as mode of second delivery. recurrence, mode of delivery

CONCLUSION: There is a significant risk of repeating labor dystocia irrespective of mode of delivery in first labor. Risk assessment regarding first labor and maternal characteristics is of great importance in counselling women in second pregnancy.

F-019

Perioperative Complications of Single Suture Versus Double Suture in Women Undergoing History Indicated Cerclage Placement. Ankit A Shah,[1] Amanda Horton.[2] *[1]Obstetrics & Gynecology, Section of Maternal Fetal Medicine, University of Chicago, Chicago, IL, USA; [2]Obstetrics & Gynecology, Division of Maternal Fetal Medicine, NorthShore University Health System - Evanston Hospital, Evanston, IL, USA.*

Objective: To compare perioperative complications between two different cerclage techniques in women undergoing a history indicated cerclage.

Methods: Retrospective cohort study of 130 women who underwent a single suture cerclage (n= 78) or double suture (n=52) history indicated cerclage. Women with a singleton pregnancy with history of cervical insufficiency whom underwent cerclage placement at < 16 weeks gestation were included. Intraoperative information and peripartum complications were chart abstracted. Student t test and mann whitney U were used for continuous variables and chi square and fisher exact test were used for categorical variables.

Results: There were no cervical lacerations or anesthesia related complications in either group. Estimated blood loss >25 ml was greater in the double suture group versus the single suture group (15.4% versus 7.8%, p=0.25. The procedure duration was longer in the double suture group than in the single suture group [median (IQR) 29 min (21.5-35) versus 25 min (17-32.7), p =0.08]. 2 (2.5%) women in the single suture group experienced vaginal bleeding and preterm premature rupture of membranes compared to 1 woman (1.9%) in the double suture group, p=0.81). Peripartum complications including preterm premature rupture of membranes, chorioamnionitis, and delivery <34 weeks were similar between groups.

Conclusion: Single suture and double suture history indicated cerclage placements are associated with low but similar rates of perioperative complications.

F-020

Intrapartum Pulse Pressure, Volume Status and Response to Epidural. David I Shalowitz,[1] Cecile Unger,[1] Altaf Saadi,[1] Kenneth Shelton,[2] Lisa Leffert,[2] Meredith Albrecht,[2] Blair Wylie.[1] *[1]Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA; [2]Anesthesia and Critical Care, Massachusetts General Hospital, Boston, MA, USA.*

BACKGROUND: Pulse pressure (PP), systolic minus diastolic blood pressure, has been proposed as a surrogate measure for intravascular volume status. In healthy adults, widened PP (>55 mmHg) is associated with hypervolemia, whereas narrowed PP (<25 mmHg) is associated with intravascular depletion. PP has not been evaluated intrapartum, when significant changes in maternal-fetal hemodynamics are possible. STUDY DESIGN: Over 60 days in 2010, hemodynamic information and response to epidural placement were collected on all women in labor that received epidural anesthesia at Massachusetts General Hospital. We investigated PP distribution and whether a narrow PP predicts the need for resuscitative measures following epidural placement, defined as a need for vasopressors or physician evaluation of fetal heart rate changes. RESULTS: 352 women met inclusion criteria. Mean PP on admission was 50 mmHg (SD 11, range 20-91) and did not vary significantly between the time of epidural placement and 30 minutes thereafter. Mean PP was wider among obese women (p=0.004) but did not differ by preeclampsia, multiple gestation, fever or spontaneous versus induced labor. 32.1% of subjects (113/352) were hyperdynamic at baseline (PP > 55mmHg) but only 1.1% (4/352) had a narrow PP (<25 mmHg). At epidural placement, 5.0% (19/352) of the women demonstrated a narrow PP (p=0.003 compared to baseline). Narrow PP did not, by itself, predict a need for resuscitation after epidural placement (p=0.77) or define a group of women at higher risk for a category 2 or 3 fetal heart tracing after epidural (p=0.11). CONCLUSIONS: Our study suggests PP is wider among laboring pregnant women than among nonpregnant adults. We recommend redefining a narrow PP during labor as <35 mmHg (10^{th} centile) and a wide PP as >65 mmHg (90^{th} centile). While a narrow PP did not predict maternal or fetal hemodynamic compromise after epidural, the effect on risk for other labor complications (dysfunctional labor, cesarean) has not yet been investigated.

F-021

Maternal and Neonatal Outcomes of Rotational Forceps Delivery: A Cohort Study. Sarah J Stock,[1] Katherine Josephs,[1] Sarah Farquharson,[1] Chris Kissack,[2] Corinne Love,[2] Sarah Cooper,[2] Jane E Norman,[1] Fiona Denison.[1] *[1]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom; [2]Simpson Centre for Reproductive Health, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.*

Objective: To determine the rates of early neonatal and maternal complications following consecutive rotational forceps deliveries.

Methods: Retrospective cohort study of consecutive cases of rotational forceps delivery performed in singleton pregnancies at 36 weeks gestation or greater, in a single tertiary referral centre in Scotland, UK between 2001 and 2008 (n=873). Comparison of outcomes associated with rotational forceps deliveries in 2008 (n=150) with those of non-rotational forceps delivery (n=873), ventouse delivery (n=159), spontaneous vertex delivery (n=3,494) and emergency caesarean delivery (n=947).

Results: There were one stillbirth associated with rotational forceps delivery but this was associated with congenital abnormality and was diagnosed before application of forceps. Following rotational forceps delivery 58/872 (6.65%) of liveborn neonates were admitted to the neontatal unit. 27/872 (3.1%) of neonates had a one or more complications that could be attributable to traumatic delivery (12 cephalohematomas, 13 nerve palsies [1 with both a nerve palsy and cephalohaematoma], 1 corneal abrasion, 1 fractured clavicle, 1 subdural haematoma). 7 babies (0.8%) had a diagnosis of neonatal encephalopathy. When one year (2008) data was compared with outcomes of other modes of delivery, neonatal admission rates after rotational forceps delivery (5/150 [3.3%]) were not significantly different from those associated with spontaneous vertex delivery (128/3,494 [3.7%; p=1.00]) or ventouse delivery (6/159 [3.8%; p=1.00]) and less than those associated with emergency caesarean section (106/947 [11.2%; p=0.002). Maternal postpartum haemorrhage rates following rotational forceps (8/150 [5.3%; p=0.008]) were lower than those associated with emergency caesarean section (142/947 [15.0%; p=0.008).

Conclusion: In contrast to previous studies that found unacceptable rates of neonatal injury, the risks of rotational forceps delivery compared to the potential benefits of avoiding caesarean section suggest there is still a place for this procedure in modern obstetric practice.

F-022

Second Thoughts on Conservative Management of Preterm Premature Rupture of Membranes beyond 32 Weeks. Ziv Tsafrir, Nadav Michaan, Gilad Margolis, Dror Mandel, Yael Salemnik, Michael Kupferminc, Sharon Maslovitz, Ariael Many. *Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel.*

Introduction: The current management of preterm premature rupture of membranes (pPROM) at 32-34 weeks of gestation is controversial. Our objective was to investigate whether conservative management at 32 weeks and beyond improves outcome.

Study design: We conducted a retrospective study of singleton pregnancies delivered between 28 and 34 weeks of gestation. The study group included patients with pPROM at gestational age of 28-34. Patients who delivered within 24 hours from pPROM were excluded from the study. The control group comprised of patients presented with spontaneous preterm delivery at 28-34 weeks gestation. Both groups were further subdivided according to gestational age – early (28-31 weeks) versus late (32-34 weeks). Adverse neonatal outcome included one of the followings: neonatal death, intraventricular hemorrhage grade 3/4, respiratory distress syndrome, periventricular leukomalacia, and neonatal sepsis.

Results*:* The study group included 94 women, and the control group included 86 women. The study group had a lower incidence of adverse neonatal outcome at the earlier weeks (28-31), compared to the control group at the same gestational age (odds ratio 0.34; 95% CI, 0.13-0.8). In contrast, at 32-34 weeks no difference in the risk for adverse neonatal outcome was noticed. Moreover, within the study group, chorioamnionitis rate was significantly higher among those who delivered at 32-34 weeks ($p<0.01$). Neonatal outcomes improved as gestational age advanced, but a long latency period between time of pPROM and delivery (>7 days) did not improve neonatal outcomes. Furthermore, higher rate of neonatal sepsis was noticed among neonates born at 32-34 weeks of gestation after a long latency period ($p=0.05$).

Conclusions*:* No advantage for conservative management of pPROM was demonstrated beyond 31 weeks. Moreover- it is possible that conservative management of pPROM at 32-34 weeks exposes both mother and neonate to infectious morbidity (chorioamnionitis, neonatal sepsis).

F-023

Description of Biological Mediator Profiles in Cervicovaginal Swabs as Markers of Progression of Normal Human Labor. Noemi Meraz-Cruz,[1] Aurora Espejel,[1] Myrna Godines,[1] Marisol Castillo-Castrejon,[1] Alejandra Migoya,[1] Marie O'Neill,[2] Felipe Vadillo-Ortega.[1] *[1]Biochemistry, School of Medicine, Universidad Nacional Autonoma de Mexico, Mexico City, DF, Mexico; [2]Environmental Health, School of Public Health, University of Michigan, Ann Arbor, MI, USA.*

Preterm labor explains almost half of preterm births, the leading cause of neonatal mortality in Latin America. Knowledge of the physiopathology of preterm labor has increased in recent years and different molecules have been identified as mediators of both normal and pathological labor. These include several cytokines, chemokines and extracellular matrix metalloproteinases. The simultaneous measurement of these compounds in samples of the micro-environments that reflect labor-associated events may be useful for identifying profiles that may be later evaluated as clinical tools for the opportune identification of women at risk for preterm labor. Objective. In this study we describe the concentrations of several compounds involved in the mechanisms of labor that were measured in cervicovaginal swabs (CVS) from women in four different stages of spontaneous labor. Methods. We obtained CVS samples from pregnant women in labor. Stage of labor was defined according to clinical characteristics going from zero activity (Stage 0) to expulsive phase (Stage 4), considering progressive changes in uterine contractions, cervical ripening and fetal membrane integrity. Samples of CVS were analyzed by Multiplex, detecting 30 compounds. Results. One hundred and seventy five CVS were analyzed at Stage 0 (n=48), Stage 1 (n=22), Stage 2 (n=34), Stage 3 (n=43) and Stage 4 (n=28). Specific change profiles were obtained using Stage 0 as the base-line comparison. The initial two stages were very similar with changes in levels of IL-1β,TNF-α,IFN-γ,MMP-8,MMP-13, and MMP-1 compared to baseline. Advanced stages were characterized by modifications in IL-1β,IL-6,TNF-α,IL-8,INF-γ,IL-2,IL-4,IL-10,IL-1RA,MMP-2, and MMP1. Discussion. Cervicovaginal secretions reflect some of the changes in the intrauterine milieu during labor. Initial stages of labor were characterized by inflammatory cytokines. Late stages were more complex, probably reflecting the activation of regulatory loops. Two clearly different profiles of these compounds were identified, corresponding to early and late stages of labor. The utility of these profiles as prognostic or diagnostic tools for normal or abnormal labor is under evaluation.

F-024

Do Non-Steroidal Anti-Inflammatory Drugs Exacerbate Postpartum Hypertension in Patients Affected with Severe Hypertensive Disorders of Pregnancy? Shane W Wasden, Ellie S Ragsdale, Stephen T Chasen, Daniel W Skupski. *Division of Maternal Fetal Medicine, Weill Cornell Medical Center, New York, NY, USA.*

OBJECTIVE: Renal function may be impaired in patients with hypertensive disorders of pregnancy (HDP), and postpartum NSAID use has the potential to adversely affect renal function. Our objective was to evaluate the association of NSAID use and postpartum hypertension (HTN) in women with severe HDP.

STUDY DESIGN: Women with severe HDP (severe gestational hypertension or severe preeclampsia) were identified through pharmacy records for magnesium sulfate administration for seizure prophylaxis at our institution from 2008-2009. NSAID use was determined and cases (NSAID exposed) and controls (no NSAID use) were included. Controls were identified through the medication administration record until a sufficient number were obtained, after which NSAID exposed patients were identified in a chronological manner during the same study period until a 2:1 ratio was achieved. The primary outcome was the mean of all postpartum mean arterial pressures (MAP) throughout the hospital stay. Secondary outcomes included: Initiation of anti-hypertensive medication (anti-HTN meds) postpartum, need for increased doses of anti-HTN meds postpartum, and adverse events related to HTN. Chi-Square test and student's T test were used for comparison, and Spearman's rho was used to evaluate correlations.

RESULTS: 204 women had severe HDP, of whom 70 (34%) were not exposed to NSAIDs and 134 (66%) were exposed. NSAID exposure was not associated with a difference in the average MAP postpartum (p = 0.5), nor any of the secondary outcomes evaluated. Exposure to NSAIDs was less likely as serum creatinine increased (p = 0.013).

	Exposed	Non-exposed	P value
Mean postpartum MAP	97 ± 7	98 ± 8	0.50
Initiation of anti-HTN medication postpartum	42/91 (46%) (91 did not initially require meds)	14/37 (38%) (37 did not initially require meds)	0.44
Increased dosage of anti-HTN medication postpartum	38/134 (28%)	17/70 (24%)	0.74
Cesarean delivery	90/134 (67%)	47/70 (67%)	1.0
Serum creatinine (mean g/dL)	0.70 ± 0.19	0.77 ± 0.20	0.013
Adverse events related to HTN	15/134 (11%)	4/70 (6%)	0.31

CONCLUSION: In women with severe HDP, NSAIDs did not appear to increase the average postpartum MAP, the rate of adverse postpartum events or increase the requirement for or dosage of anti-HTN meds. Women with more elevated serum creatinine levels were less likely to receive NSAIDs, reflecting prudent clinical management.

F-025

The Role of a Two Stitch Cervical Cerclage for Preterm Birth Prevention in Women with a History of Cervical Insufficiency. Melissa S Wong,[1] Amanda Horton,[2] Mahmoud Ismail.[1] *[1]Obstetrics and Gynecology, University of Chicago, Chicago, IL, USA; [2]Maternal/Fetal Medicine, NorthShore University Health Systems, Evanston, IL, USA.*

Objective: To determine whether placement of two stitches at the time of cervical cerclage is more effective than single stitch placement in preterm birth prevention.

Study Design: A retrospective cohort study of 124 women with a singleton pregnancy with a history of cervical insufficiency who underwent prophylactic cerclage placement at less than 16 weeks gestation. Demographic information, intraoperative findings, and medical data were chart abstracted. Statistical analysis included student t-test, chi square, Mann Whitney U test, and logistic regression. Primary outcome of interest was median gestation age at delivery.

Results: Seventy eight patients (60%) received a single stitch and fifty two patients (40%) received two stitches. Maternal characteristics were similar between the groups. There were no differences between groups with respect to gestational age at cerclage placement or type of cerclage placed. Suture type was significantly different between groups, with higher rates of Ethibond use in the two stitch group and Mersilene use in the one stitch group (p = 0.002). The procedure time was slightly longer in the two stitch group [median (interquartile range): 29 min (21.5-35) vs 25 min (17-32.7), p = 0.08]. There

was no difference in median gestational age at delivery between the one or two stitch groups [median (interquartile range): 37.0 weeks (31-39) vs 37.0 weeks (32-39), p = 0.69]. Birthweight and neonatal intensive care admission rates were similar between groups.
Conclusion: Placement of two stitches at the time of cervical cerclage does not improve gestational age at delivery when compared with single stitch placement.

F-026

Favorable Outcomes in Women with Previous Spontaneous Preterm Birth Offered Protocol-Based Prenatal Care: A Large Prospective Cohort Study. Kees WP Hollander, Maurice GAJ Wouters, Zwanique CA Tacke, Christianne MG de Groot. *Obstetrics & Gynecology, VU University Medical Center, Amsterdam, Netherlands.*
Background
Women with a history of one or more spontaneous preterm births are considered to be at high risk for recurrence. Data from randomized controlled trials have demonstrated that cervical cerclage and progesterone administration are associated with a decrease of the recurrence rate of spontaneous preterm birth.
Objective:
To prospectively evaluate the pregnancy outcome of women at high risk for recurrent spontaneous preterm birth using an evidence-based prenatal care protocol.
Methods
From April 2004 until March 2011 pregnant women with a history of at least one spontaneous preterm delivery were offered protocol-based prenatal care including first- or second trimester bacterial vaginosis screening (by vaginal culture), repeated ultrasound cervical length assesments, weekly progesterone administrations (IM, 250 mg) and supportive care by specialized nurses.
Women with a positive culture for bacterial vaginosis were treated with metronidazole medication, and women with a cervical length below 25 mm underwent a vaginal cerclage (McDonald).
Data on obstetrical and medical history, index pregnancy, delivery, maternal and neonatal outcome were prospectively collected and analyzed.
Results
In total, 389 pregnant women were prospectively followed up. Three hundred twenty-five women (83.5%) had experienced a preterm delivery in their last pregnancy, 227 (69.8%) of whom had delivered before 32 weeks.
Forty-five women were treated with metronidazole medication. Fifty-seven women underwent a secondary vaginal cerclage, 7 women had an abdominal cerclage (before pregnancy) and 7 women received a primary vaginal cerclage.
Ninety-four women (24.2%) agreed to receive weekly progesterone injections after 16 weeks (until 34 weeks).
Ninety-five women (24.4%) delivered before 37 weeks of gestational age. Thirty-two women (8.7%) had a delivery before 32 weeks of gestational age. The perinatal mortality rate (fetal death after 28 weeks and neonatal death until 4 weeks after delivery) was 3.3% (13/389).
Conclusion
Women at high risk of recurrent preterm spontaneous delivery who were offered protocol-based prenatal care had a better pregnancy outcome than would be expected from previous trials, despite a low rate of progesterone administration.

F-027

Return to Fertility after Contraception with Nomegestrol Acetate/17beta-Estradiol or Drospirenone/Ethinylestradiol. Keith Gordon,[1] Tjeerd Korver.[2] *[1]Women's Health & Endocrine, Merck & Co. Inc., Whitehouse Station, NJ, USA; [2]Women's Health & Endocrine, MSD, Oss, Netherlands.*
Objective: Nomegestrol acetate (NOMAC) combined with 17beta-estradiol (E2) is a new and highly efficacious 24/4-day monophasic oral contraceptive. Return to fertility was determined after 6 cycles of treatment with either NOMAC/E2 or drospirenone/ethinylestradiol (DRSP/EE).
Methods: This was an open-label, randomized, six-cycle study (NCT00511433) in women using NOMAC/E2 (2.5 mg/1.5 mg; 24/4-day regimen, n=32) or DRSP/EE (3 mg/0.030 mg; 21/7-day regimen, n=16). During the first post-treatment cycle (27±1 days post-treatment), ultrasonography of follicular diameter and blood sampling for determination of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and progesterone were performed every third day. Return to fertility was primarily determined by serum progesterone >16 nmol/L sustained for at least 5 days, supported by ultrasound evidence of follicular collapse and FSH/LH levels.
Results: Return of ovulation was detected within 27±1 days after last tablet intake in 22/28 (78.6%) of the women in the NOMAC/E2 group and 12/16 (75.0%) of the women in the DRSP/EE group. In 5 of 6 NOMAC/E2 treated women and the 4 DRSP/EE treated women who did not ovulate during the first post-treatment cycle, LH peaks and ultrasound evidence of imminent ovulation were observed, but progesterone had not yet reached 16 nmol/L. In 6 out of the 22 women on NOMAC/E2 with ovulation detected, the ovulation date could only be estimated within a 4-day range; these women were not included in the calculations. On average (± standard deviation), post-treatment ovulation, calculated relative to the day of last *active* tablet intake, was observed on day 20.8±4.6 in the NOMAC/E2 group and on day 20.5±3.1 in the DRSP/EE group.
Conclusion: Return to fertility after the last intake of NOMAC/E2 or DRSP/EE, as determined by onset of ovulation, occurred in the first post treatment cycle for the majority of women in both treatment groups. This is consistent with post-treatment data from two multicenter trials (NCT00511199, NCT00413062), in which return to normal menstruation within 6 weeks was observed in 849/872 (97.4%) vs 232/237 (97.9%)) women who had been on NOMAC/E2 and DRSP/EE, respectively, and who had not yet started a hormonal contraceptive and had not conceived.

F-028

Risk Factors of Surgical Evacuation Following Second Trimester Medical Termination of Pregnancy. Maarit Mentula, Oskari Heikinheimo. *Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland.*
Background: Second trimester medical termination of pregnancy (TOP) is associated with a higher risk of surgical evacuation than earlier TOP. Little is known about risk factors of surgical evacuation. Therefore, we assessed the risk factors of surgical evacuation among women un-dergoing second trimester medical TOP.
Study Design: Data on 227 women was derived from a prospective randomized trial comparing one- and two-day intervals between mifepristone and misoprostol administration in second trimester medical TOP between 2008 and 2010 (Mentula M, Suhonen S, Heikinheimo O. One- and two-day dosing intervals between mifepristone and misoprostol in second trimester medical termination of pregnancy - a randomized trial. Human Reproduction, 2011, doi: 10.1093/humrep/der218).
Results: The rate of surgical evacuation was 30.8%. The risk of surgical evacuation was increased by a history of curettage (OR 4.4; 95% CI 1.7–11.7), foetal indications for TOP (OR 6.1; 95% CI 1.1–34.4), age above 24 years (OR 2.4; 95% CI 1.1–5.3) and a two-day interval (OR 2.2, 95% CI 1.1–4.1).

Risk factors associated with surgical evacuation among the 227 women undergoing second trimester medical TOP.

Variable		OR	95%CI (confidence interval)	*p*-value	Adjusted OR*	95%CI	*p*-value
Gestation at TOP	≤16 weeks	1.07	0.59 – 1.95	0.82	1.02	0.52 – 2.00	0.96
	>16 weeks	Ref.					
Age	18 to 24 years	Ref.					
	25 to 48 years	**2.18**	**1.23 – 3.87**	**0.008**	**2.43**	**1.11- 5.34**	**0.03**
Mifepristone-misoprostol interval	One day	Ref.					
	Two days	**1.79**	**1.01 – 3.17**	**0.045**	**2.16**	**1.13 – 4.11**	**0.02**
Previous TOP**	Yes	1.02	0.58 – 1.80	0.95	0.49	0.20 – 1.22	0.13
Previous miscarriage**	Yes	1.88	0.96 – 3.68	0.07	1.34	0.59 – 3.07	0.49
Previous vaginal delivery**	Yes	1.07	0.58 – 1.98	0.84	0.53	0.23 – 1.22	0.14
Previous Caesarean section**	Yes	1.23	0.47 – 3.23	0.67	0.65	0.20 – 2.19	0.49
History of uterine curettage**	**Yes**	**2.40**	**1.33 – 4.36**	**0.004**	**4.41**	**1.66 – 11.71**	**0.003**
Indication for TOP	**Foetal**	**4.19**	**1.46 – 12.05**	**0.008**	**6.14**	**1.09 – 34.45**	**0.04**
	Other	Ref.					

Conclusions: While a history of uterine curettage and a two-day interval between mifepristone and misoprostol raised the risk of surgical evacuation in second trimester TOP special focus on these is needed in order to lower the risk of surgical evacuation in future pregnancies.

F-029

Hyperandrogenism Alters Pregnancy Rates in a Reversible Manner. Patricia T Jimenez, Antonina I Frolova, Kelle H Moley. *Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA.*
Background: Previously we demonstrated that dehydroepiandrosterone (DHEA) negatively affects ovulation and oocyte quality in mice. We have also shown that mild hyperandrogenism leads to longer estrous cycles while mice with severe hyperandrogenism do not enter estrus. In addition to effects on the ovary, DHEA and 6-aminonicotinamide (6-AN) both prevent decidualization through inhibition of the pentose phosphate pathway. The effects of DHEA on the ovary and endometrium highlight its potential as a method of contraception. Currently it is unclear if the effects of DHEA on the ovary are reversible. The purpose of this study is to evaluate the reversibility of the inhibitory effects of DHEA on the estrous cycle and pregnancy rates in a mouse model.
Materials and Methods: Three-week-old FVB/NJ female mice were fed a diet of normal chow or chow supplemented with 0.01% DHEA or 0.1% DHEA for two weeks. The mice were then mated with males of known fertility and pregnancy rates were determined. A second group was fed normal chow, 0.1%

Friday

DHEA or 0.6% DHEA for two weeks followed by normal chow for two weeks. The length of the estrous cycle and pregnancy rates were then determined.
Results: Mice on normal chow had on average 8.7±0.5 pups/litter compared to 6.5±0.9 pups/litter in the 0.01% DHEA group (p=0.05). There were no litters in the 0.1% DHEA group. Estrous cycle length in mice fed 0.1% or 0.6% DHEA followed by normal chow was similar to the controls. Furthermore, pregnancy rates in the mice fed 0.1% DHEA or 0.6% DHEA diets followed by normal chow were comparable to the controls (7.6±1, 8.5±0.7, 9.3±0.7 pups/litter, respectively).
Conclusions: Mild hyperandrogenism decreases pregnancy rates, but does not completely inhibit ovulation as in higher doses of DHEA. Reversing the hyperandrogenic state leads to resumption of normal estrous cycles and pregnancy rates. Further studies will evaluate potential abnormalities in exposed embryos and the effects of 6-AN on the ovary. The effectiveness and reversibility of DHEA support its potential as a local contraceptive.

F-030

The Effect of a Levonorgesterel-Releasing Intrauterine Device Versus Copper Containing Device on Coagulation Factors. Julia V Johnson,[1] Kristen P Wright,[2] Deborah Ikhena,[1] Xun Liao,[1] Mary Cushman,[3] Peter R Casson.[4] *[1]OB/GYN, Univ of Massachusetts, Worcester, MA, USA; [2]RSC, Lexington, MA, USA; [3]Medicine, Univ of Vermont, Burlington, VT, USA; [4]OB/GYN, Univ of Vermont, Burlington, VT, USA.*
OBJECTIVE: To determine the effect on coagulation factors of a levonogestrel-releasing intrauterine device (LNG IUD) compared to a copper containing intrauterine device (copper IUD).
METHODS: A single-blinded, randomized, controlled trial in which 36 women, following normal GYN exam and negative cultures, were randomized to either a LNG IUD (n = 15) or copper IUD (n = 21). All subjects were at least 3 months postpartum or 2 months from use of hormonal contraceptive. Blood samples were obtained at baseline, two, and four months after placement of the IUD and were analyzed for coagulation parameters, Anti-thrombin III (ATIII), D-Dimer, C-reactive protein (CRP), and total protein S, which are associated with risk of thrombosis. Study was powered to detect a 25% difference in D-Dimer and CRP. Using Mann Whitney U test for comparison, assays were compared at all time points.
RESULTS: Women in both groups had similar baseline values for the coagulation factors measured. Of note, the D-dimer value in the LNG group at 0, 2, and 4 months was 0.15, 0.14 and 0.14 respectively, contrasted with the copper group which was 0.15, 0.21 and 0.18 respectively, with a statistically significant difference in the 2-month values (p value of 0.04). There was no statistically significant difference between devices for AT III, CRP, or total protein S.

Coagluation Factors median values (range)

		Baseline	2 Months	4 Months
ATIII	LNG	103.0 (68-114)	105.5 (71-121)	102.5 (74-121)
	Copper	106.0 (89-129)	109.0 (90-123)	105.0 (91-128)
	p =	0.18	0.52	0.46
D-Dimer	LNG	0.15 (.02-.3)	0.14 (.09-.5)	0.14 (.02-.3)
	Copper	0.15 (.02-0.6)	0.21 (.05-.4)	0.18 (.05-.4)
	p =	0.31	0.04	0.13
CRP	LNG	0.79 (.18-2.8)	0.68 (.16-19.6)	0.55 (.24-2.7)
	Copper	1.27 (.37-6.2)	0.69 (.16-46.0)	0.53 (.18-4.5)
	p =	0.51	0.73	0.95
Protein S	LNG IUD	110.3 (94-136)	113.1 (85-149)	138.3 (89-137)
	Copper IUD	111.7 (68-150)	111.2 (76-133)	112.1 (81-129)
	p=	0.77	0.77	0.84

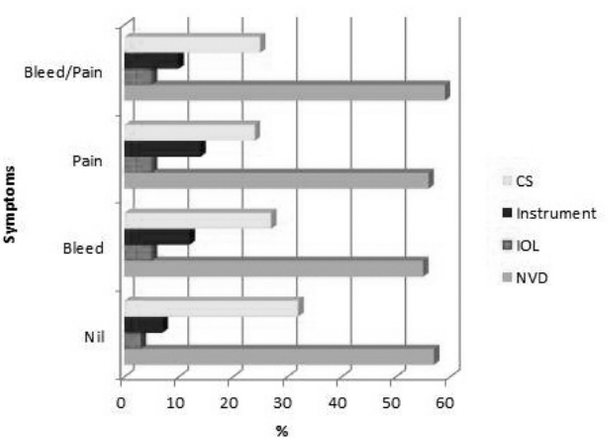
CONCLUSION: There is a transient rise in D-Dimer level 2 months after insertion of a copper IUD that is not appreciated to LNG IUD. There was no difference in coagulation factors when comparing users of LNG IUD and copper IUD over a 4-month period. This suggests no increased risk of coagulation, based on factors measures, with the use of progestin containing intrauterine device.

F-031

Reducing Risk to Women of Childbearing Potential in NIH Intramural Clinical Trials: Preventing Pregnancies and Planning for Unanticipated Pregnancies. Pamela Stratton,[1] Phoebe Oldach,[2] Emily Japp,[1] Alan Decherney,[1] Barbara I Karp.[2] *[1]PRAE, NICHD/NIH; [2]CC, NIH, Bethesda, MD.*
Introduction: Attitudes and practices regarding the inclusion of women of childbearing potential (WOCP) in clinical research have evolved in response to changing assessments of maternal-fetal health risks. To assure generation of non-gender-biased safety data from clinical trials, inclusion of WOCP has been mandated at NIH since 1993.
Materials and Methods: All Intramural on-site clinical trials that included WOCP and posed more than minimal risk were reviewed. Data were collected from the protocol (P), consent (C), and, when applicable, assent, about the 1) risks the interventions posed, using FDA pregnancy drug category (A, B, C, D, X, Unknown) 2) trial phase (0 to IV) 3) NIH institute 4) duration of intervention use (≤ or > 1 month) and 5) methods employed for protecting WOCP, including pregnancy testing, contraception use, and plans for unanticipated pregnancies. Information was compared across documents.
Results: 492 of 1640 studies met inclusion criteria with 80% posing long-term risk (>1 month). 67% were sponsored by NCI, NHLBI, or NIAID; and 93% were early phase trials (phase 0, I, or II). Overall, 90% studied FDA pregnancy category C or worse agents with 44% category C or D, 9% category X, and 38% unknown (not yet FDA approved). Minors participated in 29% of studies. Pregnancy testing was included in 95% of studies (P:93%; C:78%), but 60% of studies lasting >one month did not specify its frequency. Nearly 90% lasting >one month required contraception use, with contraception more frequently suggested in consent than protocol (C:83% vs P: 80%). While 94% considered pregnancy, 60% mentioned pregnancy only in protocol study exclusion criteria, lacking discussion of how study intervention would be managed if pregnancy occurred during the trial. 62 protocols and 123 consents had a pregnancy plan not conveyed in the other document.
Discussion: Most NIH trials including WOCP were early phase studies and used high risk FDA pregnancy category agents for at least a month. A standardized approach would enhance safe inclusion of women of childbearing potential in clinical trials. Efforts to consider include: improving the consistency of information across trial documents, clearly stating pregnancy testing intervals, requiring contraception, and informing women about plans if pregnancy occurs while on study intervention.
Funding: Intramural Program NICHD and CC, NIH

F-032

The Influence of Symptoms at Presentation to an Early Pregnancy Assessment Unit (EPAU) and Mode of Delivery. Katerina N Bambang, David G Lambert, Justin C Konje. *Cancer Studies and Molecular Medicine, University of Leicester, Leicester, East Midlands, United Kingdom.*
Introduction: Women presenting to EPAU with symptoms of threatened miscarriage in the first trimester are at greater risk of adverse maternal and perinatal outcome including antepartum haemorrhage. This is particularly so in women presenting with bleeding. The aim of this study was to assess the relationship between symptoms at presentation in EPAU and mode of delivery.
Methods: 332 healthy pregnant women between 6-12 weeks gestation were recruited at presentation to the EPAU. Their symptoms as well as a range of demographic factors were noted and they were then followed up to delivery. The women were divided into those with no symptoms, those presenting with pain, bleeding or both pain and bleeding.
Results: 283 (85%) of the women had a live birth. The rate of normal vaginal delivery was approximately the same for all four groups ranging from about 55-59%. Women presenting in the first trimester with symptoms of pain were at higher risk of having an instrumental delivery when compared to women who were asymptomatic at presentation (p=0.001). Interestingly, this was reversed when looking at Caesarean section but this finding was not statistically significant.



Conclusion: Women presenting to EPAU with symptoms of pain are at higher risk of intervention than women who are asymptomatic in the first trimester. This information may be useful when risk assessing women during the antenatal period.

F-033

Closure of Hospitals with Acute Obstetric Care in The Netherlands: Effects on Perinatal Mortality in a System with High Numbers of Intrapartum Referrals and Heterogeneous Hospitals. Gouke J Bonsel,[1,2,3] Jashvant Poeran,[1] Hanneke P De Graaf,[1] Gerard JJM Borsboom,[2] Erwin Birnie,[4] Eric AP Steegers,[1] Johan P Mackenbach.[2] *[1]Obstetrics and Gynaecology, Erasmus MC, Netherlands; [2]Public Health, Erasmus MC, Netherlands; [3]Rotterdam Midwifery Academy, , Netherlands; [4]Institute of Health Policy and Management, Erasmus University, Netherlands.*

BACKGROUND The Netherlands has a high perinatal mortality. Organizational factors such as staffing levels and insufficient 7*24h acute clinical obstetric care have been shown to be contributing factors. Consequently, the Dutch Ministry of Health suggested a possible closure of a number of hospitals providing acute obstetric care. In most cases, however, closure will result in increased travel time to a hospital. Furthermore, previous results showed substantial inter-hospital variation regarding organizational factors.

METHODS Data on perinatal outcomes were obtained from The Netherlands Perinatal Registry (2000-2008, n=1,584,800 single pregnancies). Organizational details were obtained from each hospital by survey. First, a comprehensive multilevel regression model was created for all singleton hospital deliveries, taking into account both patient factors (as casemix) and several care factors. Care factors were type of delivery (elective vs. non-elective), travel time (interacting with risk status), and a set of organizational features. Second, two scenarios were defined with 10 hospitals hypothetically closed according to different principles: (1) smallest first, (2) smallest first but avoiding adjacent closures. The regression model was applied to predict intrapartum and early neonatal mortality among women changing to another hospital. RESULTS Scenario 1 resulted in doubled travel time, and 10% increased mortality (0.34% vs. 0.38%). In scenario 2 mortality was slightly decreased (0.33% vs. 0.32%), with less effect on travel time. Heterogeneity of hospitals was striking causing simultaneous improvement and deterioration of mortality depending on the features of the nearest alternative hospital. Furthermore, high risk groups suffer more from travelling and gain more if a specialized hospital is nearby.

CONCLUSION The optimal strategy for merging obstetric care facilities should account for patient- as well as organizational features of both intended closing and 'surviving' hospitals. Our framework may be of wider utility as hospital merging is considered in other countries as well.

F-034

The Epidemiology of Preterm Birth < 34 Weeks at a Tertiary Care Academic Medical Center. Brendan D Connealy,[1] Carlos A Carreno,[1] Benjamin A Kase,[1] Jerrie S Refuerzo,[1] George Saade,[2] Sean C Blackwell.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Science Center, Houston, TX, USA; [2]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective:

In order to effectively implement preterm birth (PTB) prevention strategies, a better understanding of the current epidemiology of PTB is needed. Our purpose was to 1) characterize the frequency and sub-types of early PTB in both low risk and high risk populations and 2) estimate the relative contribution from each population to the overall early PTB rate.

Study Design:

All PTBs 20 wks 0 days to 33 wks 6 days at a tertiary care hospital over a one year period (Jan 1-Dec 31, 2010) were studied. Early PTBs < 34 weeks were categorized as spontaneous (PTL or PPROM), medically indicated (MI), or non-medically indicated (NMI). The frequency and subtype of early PTB were characterized for both low risk and high risk populations. Low risk population was defined as singleton nulliparous and multiparous women without a prior PTB < 37 wks. High risk was defined as singleton pregnancy with prior PTB < 37 weeks or women with multiple gestations. Exclusion criteria were antepartum stillbirth (IUFD) and pregnancies with major congenital anomalies.

Results:

Over the study period there were 5,082 total births and 395 (7.8%) delivered < 34 wks. Of those < 34 wks, 58 were excluded (43 IUFD, 15 anomalies) for a total n=342. The proportion of various PTB subtypes were similar across gestational age (GA) ranges.

Frequency of PTB subtype across GA ranges 20-34 wks

	Gestational Age (wks)				
PTB Subtype	20-24 (N=19)	24-28 (N=75)	28-32 (N=113)	32-34 (N=134)	P-value
sPTL	13 (68%)	32 (43%)	43 (38%)	62 (46%)	0.08
PPROM	3 (16%)	16 (21%)	17 (15%)	14 (10%)	0.21
MI	3 (16%)	27 (36%)	53 (47%)	58 (44%)	0.54

There were no cases of NMI early PTB. Low risk women (singleton nulliparous or multiparous with no prior PTB) accounted for 45% of the overall early PTB's. Of high risk women, those with a prior PTB had an early PTB rate of 13% and accounted for 20% of the overall PTBs. Women with multiple gestations had an early PTB rate of 32.4% and accounted for 35% of the overall PTBs.

Conclusions:

In our population, screening programs that only target high risk women would fail to identify nearly half of women destined for PTB < 34 wks.

F-035

Maternal Obesity Is Associated with Increased Maternal and Offspring Morbidity in Both Primiparous and Multiparous Women. FC Denison,[1] GS Scotland,[2] P Norwood,[2] TA Mahmood,[3] C Morris,[4] JE E Norman,[1] S Bhattacharya,[5] A Raja,[6] A Lee.[6] *[1]MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom; [2]Health Economics Research Unit, University of Aberdeen, United Kingdom; [3]Forth Park Hospital, Kircaldy, NHS, United Kingdom; [4]Information Services Division, NHS, United Kingdom; [5]Obstetrics and Gynaecology, University of Aberdeen, United Kingdom; [6]Division of Applied Health Sciences, University of Aberdeen, United Kingdom.*

The aim of the study was to determine the effect of maternal body mass index (BMI) on maternal/offspring clinical outcomes using national health service (NHS) for Scotland. Data on all women with singleton pregnancies who delivered in Scottish maternity units from 1st Jan 2003 until 31st Dec 2009 were included. After excluding pregnancies where maternal BMI was indeterminable, the final dataset comprised 124,280 deliveries from 109,692 women. Women were grouped according to BMI by WHO criteria with BMI 20<25kg/m^2 used as the reference group. Analyis included descriptive statistics, multiple linear regression (primiparous analysis) and random effects hierarchical regression (all pregnancies analysis). A total of 76.3%, 64.1%, 74.9% and 76.3% of women with a normal, overweight, obese and morbidly obese BMI in one pregnancy remained in the same BMI group in their next pregnancy. After adjusting for maternal age, deprivation and smoking, overweight, obese and morbidly obese primiparous and multiparous women had an increased risk of pregnancy induced hypertension, pre-existing diabetes, gestational diabetes, other maternal diseases, induction of labour, elective and emergency caesarean and iatrogenic preterm birth compared to women with a normal BMI (all p<0.05). The average birthweight of babies born to primiparous and multiparous women was lower in underweight women (180g; 181g) and higher in overweight (99g; 104g), obese (129g; 155g) and morbidly obese (121g; 180g), respectively (all p<0.001). For women with a normal BMI in earlier pregnancies, becoming obese and morbidly obese in subsequent pregnancies was associated with increased risk of induction of labour, and caesarean section and neonatal unit admission >48 hours, respectively (all p<0.05). In conclusion, maternal obesity has major consequences for maternal and offspring health outcomes for primiparous and multiparous women. At a national level, the impact of maternal obesity should be considered when considering service delivery.

F-036

Maternal Obesity Is Independently Associated with Increased Antenatal Admissions and Health Service Costs. FC Denison,[1] AJ Lee,[2] C Morris,[3] TA Mahmood,[4] JE Norman,[1] A Raja,[2] S Bhattacharya,[5] P Norwood,[6] GS Scotland.[6] *[1]MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom; [2]Division of Applied Health Sciences, University of Aberdeen, United Kingdom; [3]Information Service Division, NHS, United Kingdom; [4]Forth Park Hospital, Kircaldy, NHS, United Kingdom; [5]Obstetrics and Gynaecology, University of Aberdeen, United Kingdom; [6]Health Service Research Unit, University of Aberdeen, United Kingdom.*

The aim of the study was to determine the effect of maternal body mass index (BMI) on inpatient costs for the health service in Scotland. Data on all women with singleton pregnancies who delivered in Scottish maternity units from 1st Jan 2003 until 31st Dec 2009 were included. After excluding pregnancies where maternal BMI was indeterminable and costs could not be estimated, the final dataset for analysis was 285,361 admissions (n=123,931 pregnancies in 109,291 women across 45 hospitals). Duration of stay, hospital, and provider data were combined with hospital level specialty unit cost data to estimate the cost of each admission. Women were grouped according to BMI by WHO criteria with BMI 20<25kg/m^2 used as the reference group. Data were analysed by ANOVA and Generalized Linear Modelling. After adjusting for age, deprivation, hospital and smoking status, high and low BMI was significantly associated with increased maternal inpatient costs. Additional costs associated with being underweight, overweight, obese and morbidly obese were £171, £157, £874 and £865 (UK), respectively. After adjusting for covariates, overweight, obesity,

and morbid obesity were associated with a 16%, 45% and 88% increased risk of hospital admission antenatally or postnatally ($p<0.01$) . Underweight, overweight, obesity and morbid obesity were associated with a 5%, 7%, 17% and 30% increase in the total number of admission days compared with normal weight ($p<0.01$). To further understand the relationship between maternal hospital costs and BMI, sensitivity analysis was performed. Inclusion of year suggested changes in obstetric practices over the duration of the study with costs decreasing across all BMI categories, whilst adjustment for obstetric indicators including gestational diabetes and caesarean section demonstrated that the U-shaped relationship between BMI and costs remained. In conclusion, maternal obesity has significant economic implications for delivery of maternity services independent of co-morbidities and mode of delivery.

F-037

The Influence of Parental Biometrics & Smoking on Neonatal Body Fat Deposition. Fred A English,[1] Louise C Kenny,[1] Fergus P McCarthy,[1] Mairead Kiely,[2] Deirdre M Murray,[3] Jonathon O Hourihan,[3] Ali S Khasahan.[1] *[1]Anu Research Centre, University College Cork; [2]School of Food & Nutritional Sciences, University College Cork; [3]Paediatrics & Child Health, University College Cork, Ireland.*

BACKGROUND

Over the last 20 years the number of obese children has doubled with recent figures suggesting 48% of children in the United States have a body mass index (BMI) above the normal range[1]. It is possible that susceptibility to obesity is determined in-utero or even before conception. Obese children have greater propensity to become obese adults and endure the accompanying morbidities[2]. The aim of this study was to determine the effect of parental biometrics and smoking behaviour on neonatal percentage body fat.

METHODS

As part of a large birth cohort study neonatal percentage body fat was measured within the first 4 days of life using air-displacement plethsymography (n=955). The cohort excluded admissions to neonatal intensive care. Multivariate linear regression in SPSS was used to analyse the effect of paternal and maternal; birthweight, BMI, 4-site skin fold determined % body fat and smoking on neonatal percentage body fat.

RESULTS

Mean neonatal body fat was calculated at 11.1% ± 4.1%. Maternal obesity (30-35kg/m²) significantly increased neonatal body fat (Mean difference 1.56%, (95% CI 0.67, 2.44)). Increasing paternal birth weight (PBW) was associated with increased neonatal body fat deposition (Mean difference 0.5%/KgPBW, (95% CI 0.05, 0.95). Maternal birthweight, maternal smoking, paternal smoking and 4-site skin fold determined body fat % had no effect on neonatal body fat percentage

INTERPRETATION

Increased neonatal body fat has far reaching consequences. This study is the first to utilise neonatal plethsymography and a robust statistical model to probe the parental characteristics effecting neonatal body fat deposition. Our study clearly links maternal obesity with an increase in neonatal fat percentage. Identifying additional parental factors affecting neonatal body fat percentage may help identify fetuses at risk of both large and small for gestational age and lessen subsequent perinatal complications.

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F-038

The Effect of Maternal Prenatal Stress, Anxiety and Depression on Neonatal Biometric Measurements. Claire M Everard,[1] Ali S Khashan,[1] Lesley M McCowan,[2] Robyn A Morth,[3] Gus A Dekker,[4] Claire T Roberts,[4] Louise C Kenny,[1] on Behalf of the SCOPE Consortium. *[1]Anu Research Centre, University College Cork, Cork, Ireland; [2]Obstetrics & Gynaecology, University of Auckland, Auckland, New Zealand; [3]Division of Women's Health, King's College, London, United Kingdom; [4]Obstetrics & Gynaecology, University of Adelaide, Adelaide, Australia.*

Background: Maternal psychological stress has been linked with higher risks of adverse outcomes during pregnancy including fetal growth restriction. We therefore hypothesized that high stress, anxiety and depression scores early in pregnancy would be associated with decreased neonatal biometric measurements.

Methods: The study cohort consisted of 3531 nulliparous low risk women who participated in the SCOPE (SCreening for Pregnancy Endpoints) study. Psychological state was assessed using the Perceived Stress Scale (PSS), the State Trait Anxiety Inventory (STAI) and the Edinburgh Postnatal Depression Scale (EPDS) at approximately 15 and 20 weeks' gestation. We compared neonatal head circumference, midarm circumference and length in women who had scores in the lowest quartile compared with the other three quartiles for stress and anxiety. For the depression analysis, the cohort was divided into three groups, where the two highest groups (moderate and high scores) were compared to the lowest score group. Multivariate linear regression was used for data analysis adjusting for maternal age, body mass index, smoking, alcohol intake and ethnic origin.

Results: The average head circumference in the cohort was 34.68 cms, (SD 2.02) mid arm measurement was 10.73 cms (1.18) and length 50.17 cms (2.87). The estimates supported an association between high maternal depression (Table 1) but not stress and anxiety at 20 weeks gestation and neonatal measurements (data not reported here). Furthermore, there was no relationship between stress, anxiety and depression scores at 15 weeks' gestation and neonatal measurements.

Depression scores at 20 weeks' gestation and biometric measurements

EPDS	Head Circumference	Length	Mid arm Circumference
Low Score (0-4)	Reference		
Moderate Score (5-9)	0.13 (-0.20, 0.28)	0.21 (-0.04, 0.45)	0.09 (-0.01, 0.19)
High Score (10-25)	0.19 (0.01, 0.37)	0.17 (-0.12, 0.47)	0.14 (0.20, 0.03)

Conclusion: We found limited evidence to support our hypothesis that prenatal psychological stress is associated with reduced fetal growth. Further analyses will be performed using the final SCOPE cohort of approximately 6000 participants.

F-039

Associations between Perinatal Hemorrhage and Acute Renal Failure: Race/Ethnicity Disparity. Darios Getahun,[1] Michael J Fassett,[2] Deborah A Wing,[3] Steven J Jacobsen.[1] *[1]Department of Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA, USA; [2]Department of Obstetrics & Gynecology, Division of Maternal-Fetal Medicine, Kaiser Permanente West Los Angeles Medical Center, Los Angeles, CA, USA; [3]Department of Obstetrics & Gynecology, University of California Irvine, Orange, CA, USA.*

OBJECTIVE: To evaluate the association between perinatal hemorrhage and acute renal failure (ARF) based on maternal race/ethnicity.

METHODS: In this retrospective cohort study we studies 544,757 women with singleton pregnancies who were delivered at ≥20 weeks of gestation in KPSC hospitals between 1991 and 2009. We used birth certificate and ICD-9 codes from hospitalization and outpatient encounters to examine the association between gestational hemorrhages with ARF) and whether the risk varies by maternal race/ethnicity. Multivariable logistic regression models were used to estimate the adjusted odds ratios (OR) and their 95% confidence intervals (CI).

RESULTS: Rates of gestational hemorrhages among non-Hispanic White (NHW), Black, Hispanic, and Asian/Pacific Islander (A/PI) women were 3.7%, 3.9%, 4.2%, and 5.3%, respectively ($p<0.01$). There were 132 (0.02%) cases of ARF identified during the study period. Women with gestational hemorrhages were more likely to have gestational diabetes ($p<0.01$) and preterm delivery ($p<0.01$). Hemorrhage in term-gestation was significant associations with ARF among Blacks (OR 12.6, 95% CI 1.5, 105), Hispanics (OR 12.3, 95% CI 2.8-54.8), and A/PIs (OR 44.7, 95% CI 10.8-185)], but not among Whites (OR 9.1, 95% CI 0.9-95.8). Hemorrhage at a preterm-gestation was significantly associated with increased risk of ARF among A/PIs. Compared with uncomplicated pregnancy, perinatal hemorrhage requiring cesarean delivery conferred a 3.3-fold (95% CI 1.5-7.6) increased odds of ARF. This risk was even higher in women who had hemorrhage requiring hysterectomy (OR 47.3 95% CI 12.0-186.9).

CONCLUSION: Perinatal hemorrhage is significantly associated with ARF and the pattern of risk varied according to race/ethnicity and gestational age at delivery. The identification of women at risk for ARF may help obstetricians closely monitor their patients.

F-040

Racial and Ethnic Differences in Trends in Induction of Labor. Darios Getahun,[1] Michael J Fassett,[2] Sascha Dublin,[3] Deborah A Wing,[4] Aeron B Caughey,[5] Tefera Gezmu,[6] Steven J Jacobsen.[1] *[1]Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA, USA; [2]Obstetrics & Gynecology, Division of Maternal-Fetal Medicine, West Los Angeles Kaiser Permanente Southern California, Los Angeles, CA, USA; [3]Group Health Research Institute, Group Health Cooperative, Seattle, WA, USA; [4]Obstetrics & Gynecology, University of California Irvine, Orange, CA, USA; [5]Obstetrics & Gynecology, Oregon Health and Science University, Portland, OR, USA; [6]Epidemiology, University of Medicine and Dentistry-School of Public Health, Piscataway, NJ, USA.*

OBJECTIVE: To characterize differences in trends for induction of labor (IOL) according to maternal age and race/ethnicity.

METHODS: Data came from Kaiser Permanente Southern California's (KPSC) Perinatal Service System and Hospital Inpatient records, which include births in all KPSC hospitals between 1997 and 2008 (n= 330,000). IOL was defined as present if IOL was checked on the birth certificate or if ICD-9-CM codes for IOL were coded in the health plan record. We further examined differences by race/ethnicity and age by comparing the proportion of births initiated by IOL in the earliest (1997-98) versus most recent (2007-08) biennial periods. Maternal race/ethnicity was categorized as Non-Hispanic White (White), Non-Hispanic Black (Black), Hispanic, and Asian/Pacific Islander (Asian/PI). Adjusted relative risks (RR) and their confidence intervals (CI) comparing the two time periods were derived from Poisson regression models.

RESULTS: The proportion of births initiated by IOL increased from 14.3% in 1997 to 25.1% in 2008 (p-value for linear trends <0.0001). From 1997-98 to 2007-08, this proportion increased among Whites (18.4% to 29.7%; RR 1.8 [95% CI 1.7-2.0]), Hispanics (13% to 24.3%; RR 2.1 [95% CI 1.9-2.2]), and Asian/PI (12.8% to 22.3%; RR 1.9 [95% CI 1.7-2.1]). Among Blacks, IOL increased from 12.2% in 1997-98 to 29% in 2007-08 (RR 2.9 [95% CI 2.6-3.3]), largely driven by an increase in women under 25 years or from 25-34 years of age. The increases were similar according to maternal race/ethnicity categories in women ≥35 years old.

CONCLUSION: This study shows that the proportion of births initiated by IOL significantly increased during the study period, with the largest increase among Black women. The disproportionately rising trend in IOL among Black women is of concern and deserves further investigation.

F-041

Maternal Asthma Is a Significant Contributor to Neonatal Morbidity. Nicolette A Hodyl,[1] Wendy Scheil,[2] Michael J Stark,[1] Vicki L Clifton.[1] *[1]Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adelaide, SA, Australia; [2]Epidemiology Unit, South Australian Department of Health, Adelaide, SA, Australia.*

Background: Asthma is the most prevalent chronic condition to affect pregnancies in Australia, currently affecting 12% of pregnant women and expected to rise to 20% in the next five years. We have previously reported an association between maternal asthma and adverse perinatal outcomes, including pre term delivery, still birth and intrauterine growth restriction (IUGR), and other studies suggest and increased risk for congenital malformations. This study aimed to examine the effect of asthma during pregnancy on perinatal birth outcomes and congenital malformations rates in a South Australian cohort.

Method: All singleton birth outcomes in South Australia over ten years (1999-2008: n=178,000) were analysed to assess the affect of asthma on perinatal outcomes. Logistic regression was used to calculate odds ratios and adjust for factors including smoking, maternal age and degree of prematurity.

Results: Asthma was reported in 6.5% of pregnancies and was associated with a 27% increased risk of preterm delivery (95% CI 1.19-1.36). This effect remained after adjusting for maternal smoking, parity, maternal age and gestational diabetes (OR=1.21, 95% CI 1.13-1.30) Congenital abnormalities were more frequent in pregnancies associated with asthma (3.9% versus 2.4% P<0.001). An increased requirement for resuscitation (OR=1.15, 95% CI 1.08-1.23) and oxygen therapy >4 hours (OR=1.12, 1.03-1.22) was also observed in pregnancies associated with asthma after adjusting for preterm birth, explaining the significant increase in neonatal intensive care admission rates (2.8% versus 2.2%; P<0.001).

Conclusion: An increased risk of preterm delivery and congenital malformations were observed in pregnancies complicated by asthma, importantly, resuscitation and oxygen therapy were required by neonates of mothers with asthma irrespective of the degree of prematurity. This study has therefore highlighted maternal asthma as a significant contributor to neonatal morbidity.

F-042

Pregnancy Outcomes in the South Australian Indigenous Population: The Effect of Asthma during Pregnancy. Nicolette A Hodyl,[1] Wendy Scheil,[2] Vicki L Clifton.[1] *[1]Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adelaide, SA, Australia; [2]Epidemiology Unit, South Australian Department of Health, Adelaide, SA, Australia.*

Background: While asthma has been recognised as a significant contributor to maternal and neonatal adverse outcomes in pregnancy, the impact of asthma on pregnancy outcomes amongst the indigenous Australian population is unknown. Given that asthma rates are significantly higher in indigenous than non-indigenous Australians, We hypothesised that adverse outcomes associated with asthma would be greater in this population compared to non-indigenous Australians.

Method: All birth outcomes in South Australia over ten years (1999-2008) were analysed to assess the effect of asthma on perinatal outcomes. Rates of adverse maternal and neonatal outcomes with asthma were compared between the indigenous (n=4995) and non-indigenous populations (n=173,005).

Results: An average of 2.7% of all new mothers in South Australia identified as aboriginal and/or Torres Strait Islander descent, with a gradual increase from 2.4% in 1999 to 3.1% in 2008. The reported prevalence of asthma during pregnancy in indigenous Australians (7.8%) was higher than in non-indigenous Australians (6.4%: P<0.001). Within the indigenous population, asthma was associated with higher rates of pre-existing diabetes and essential hypertension compared to the non-asthmatic population; both of which are 50% greater than corresponding rates in non-indigenous Australians (P,0.01). Rates of Preterm birth, small for gestational age deliveries, IUGR, still births, neonatal intensive care admissions, requirements for resuscitations and oxygen therapy (>4 hours) were all significantly greater in the indigenous compared to the non-indigenous populations (P<0.01 for each). The higher prevalence of these adverse neonatal outcomes was not further affected by the presence of asthma within the indigenous population.

Conclusion: An increased likelihood of asthma with co-morbidities including pre-existing hypertension and diabetes during pregnancy were observed in the indigenous population. The effects of asthma on neonatal outcomes in the indigenous compared to the non-indigenous Australian population are not distinguishable, most likely due to the disturbingly high rates of adverse outcomes observed within this population irrespective of asthma.

F-043

Rates of Maternal Complications of Pregnancy Are Increased with Asthma: A Ten-Year Analysis. Nicolette A Hodyl,[1] Wendy Scheil,[2] Vicki L Clifton.[1] *[1]Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adelaide, SA, Australia; [2]Epidemiology Unit, South Australia Department of Health, Adelaide, SA, Australia.*

Background: In Australia, the highest rates of asthma are observed in females of reproductive age (14%). While asthma during pregnancy has been associated with poor outcomes, other studies have reported an absence of such effects. This discrepancy may be due to limited sample sizes or the confounding effect of maternal smoking, which is a major co-morbidity in pregnant asthmatic populations. Smoking itself is well known to complicate pregnancies, but can also lead to asthma exacerbations, which are also independently associated with poor pregnancy outcomes. The aim of this study was to examine the effect of asthma during pregnancy, adjusting for smoking, on prenatal outcomes in a large South Australian cohort.

Method: A retrospective analysis of all singleton pregnancy and birth outcomes in South Australia over ten years (1999-2008; n=178,000) was undertaken to assess the effect of asthma on maternal pregnancy outcomes. Logistic regression was used to calculate odd ratios and adjust for factors including smoking.

Results: Asthma was reported in 6.5% of SA birth records; a rate lower than the state asthma prevalence of 14%. After adjusting for confounds including smoking, asthma was associated with an increased risk of pregnancy associated hypertensive disorder (odds ratio = 1.36, 96%CI 1.28-1.46), antepartum haemorrhage (OR = 1.31, 95%CI 1.2-1.44), urinary tract infection (OR = 1.48, 95%CI 1.34-1.63), caesarean section (OR = 1.22, 95%CI 1.17-1.27) and gestational diabetes (OR = 1.32, 95%CI 1.20-1.44).

Conclusion: While asthma appeared under-reported in this population, increased risk of maternal complications were clearly evident in pregnancies complicated by asthma, irrespective of smoking. This study has confirmed previous reports of increased adverse outcomes in pregnancies complicated by asthma, indicating that asthma should be addressed and managed appropriately as part of a routine antenatal care.

F-044

Increased Risk of Miscarriage and Ectopic Pregnancy in Patients with Irritable Bowel Syndrome. Ali S Khashan,[1] Eamonn MM Quigley,[2] Roseanne McNamee,[3] Fergus P McCarthy,[1] Fergus Shanahan,[2] Louise C Kenny.[1] *[1]Anu Research Centre, University College Cork, Cork, Ireland; [2]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland; [3]Biostatistics Group, University of Manchester, Manchester, United Kingdom.*

Background: Irritable bowel syndrome (IBS) is the most commonly diagnosed gastrointestinal condition. The condition is most prevalent in women of reproductive age. To date the impact of IBS on pregnancy outcome has not been investigated. The objective of the present study was to investigate the impact of maternal IBS on the risk adverse of pregnancy outcome.

Methods: A UK General Practice Research Database (GPRD) study was conducted. The database contains demographic information, major diagnoses and medical events for more than 10 million people from general practitioners, referrals to specialists and hospital admissions. Several IBS studies and several pregnancy studies have used GPRD data in the past two decades. The study cohort consisted of 100,000 women selected by stratified random sampling from 3.7 million women with a diagnosis of pregnancy between 01/01/1990 and 31/12/2008. Women were defined as exposed if they had a recorded diagnosis of IBS before their pregnancy. Outcome measures were spontaneous miscarriage, ectopic pregnancy, pre-eclampsia and stillbirth. Odds Ratios and 95% confidence intervals of the association between IBS and the outcome measures were estimated using logistic regression adjusted for maternal age, body mass index, smoking, social deprivation and other co-morbidities. Sensitivity analyses were performed to assess the effect of maternal age and smoking on the observed associations.

Results: Among the study cohort of 100,000 women, 26,543 women had a diagnosis of IBS before their pregnancy. Maternal IBS was found to be associated with a moderately increased risk of spontaneous miscarriage (adjusted OR=1.2, [95% CI: 1.1-1.3]) and ectopic pregnancy (adjusted OR=1.3, [95% CI: 1.1-1.6]). There was no evidence to suggest an association between IBS and pre-eclampsia (adjusted OR=1.1, [95% CI: 0.8-1.4]) or stillbirth (adjusted OR=1.0, [95% CI: 0.7-1.4]).

Conclusion: These findings suggest that maternal IBS, a disorder that is common in women of reproductive age, may increase the risk of spontaneous miscarriage and ectopic pregnancy. This is the first study to demonstrate an association between IBS and adverse early pregnancy outcome and the strength and nature of this association warrants further research.

F-045

The Use of Crown Rump Length To Predict Small or Large for Gestational Age and Preterm Birth. Catherine T Cronin,[1] Ali S Khashan,[1] Fergus P McCarthy,[1] Keelin O'Donoghue,[1] Robyn A North,[2] David I Broadhurst,[3] Philip N Baker,[3] Gustaaf A Dekker,[4] Lucilla Poston,[2] Louise C Kenny.[1] *[1]Anu Research Centre, University College Cork, Cork, Ireland; [2]Obstetrics and Gynaecology, King's College London, London, United Kingdom; [3]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada; [4]Obstetrics and Gynaecology, Adelaide University, Adelaide, Australia.*

Background: First trimester growth disorders can persist throughout pregnancy and previous studies have identified that reduced Crown Rump Length (CRL) is associated with small for gestational age (SGA) and preterm delivery (<37 weeks gestation) (1). Our aim was to test this association in a large prospective cohort study. In addition we investigated the association between CRL and large for gestational age (LGA).

Methods: 3531 nulliparous women were recruited to the multicentre prospective Screening for Pregnancy Endpoints (SCOPE) study, between November 2004 and August 2008. 2003 patients from the original cohort were included in this analysis, having a normal menstrual history, certain Last Menstrual Period, and CRL measuring within +6 and -6 days of that expected for gestational age. We examined the difference in expected and measured CRL in relation to subsequent growth disorder and preterm birth. First trimester CRL was divided into three groups with CRL; 1) below -1 day from expected considered growth restricted; 2) above +1 day considered macrosomic in the first trimester; and 3) between -1 day and + 1 day used as the reference group. Logistic regression analysis in SPSS was used for data analysis.

Results: CRL was not associated with SGA, LGA or preterm delivery after adjusting for maternal age, smoking status, alcohol and BMI (Table 1).

CRL Difference in the First Trimester and Pregnancy Outcome

CRL difference	SGA, OR (95% CI)	LGA, OR (95% CI)	Preterm Birth, OR (95% CI)
-1 to +1 days	ref[1]	ref[1]	ref[1]
-6 to -2 days	1.0(0.6, 1.6)	1.3(0.7, 2.1)	1.0(0.5, 2.1)
+2 to + 6 days	0.8(0.5, 1.2)	1.3(0.9, 2.0)	0.7(0.3, 1.4)

Conclusions: We found no evidence for an association between first trimester CRL and risk of SGA or LGA and preterm delivery. These findings are in contrast to previous papers that provided evidence for an association. We currently await the remaining SCOPE data, which approaches 5690 in total, for complete and more detailed analysis.

References:

1) Smith et al., NEJM, 1998; 339: 1817-1822

F-046

Antecedents of Success in External Cephalic Version. Joel D Larma. *Obstetrics & Gynecology, Division of Maternal Fetal Medicine, The Ohio State University, Columbus, OH, USA.*

OBJECTIVE:

To determine the antecedents of successful external cephalic version (ECV) among those undergoing an attempt at ECV in a large population-based birth registry.

STUDY DESIGN:

This is a population based retrospective cohort study of all birth records using the National Health Center for Vital Statistics 2008 Natality Public Use File. The primary outcome was successful external cephalic version and the primary predictor variables are demographic variables listed in the Table. Initial univariate analysis was performed using Chi-square or Fisher's exact tests for categorical variables and the t-test or Mann-Whitney U test for continuous variables. A multiple logistic regression model was performed using predictor variables that were either chosen a priori or were found to alter the primary OR by 10-15%. Goodness of fit was assessed using the Hosmer-Lemeshow test. All analyses were performed using STATA 11.0.

RESULTS:

There were 4,255,156 births included in the final analysis. The total number of successful external cephalic versions was 8,257 while the total number of failed external cephalic versions was 2,738. The success rate for ECV among this population was 75.10%. The following variables were found to be significantly associated with success of ECV: maternal age, Asian race, paternal age, early prenatal care. The following variables were found to be significantly associated with failure of ECV: maternal diabetes, male gender of fetus, large birthweight of infant, prior cesarean section and poor maternal weight gain. (See Table) The Hosmer-Lemeshow test was nonsignificant indicating a good fit of the model.

OR for Failure of ECV	
	aOR (95% CI)
Black Race	0.98 (0.70, 1.36)
Maternal Age (continuous in years)	0.97 (0.95, 0.98)
Unmarried	1.08 (0.94, 1.26)
Paternal Age (continuous in years)	0.98 (0.98, 0.99)
Maternal Weight gain (continuous in pounds)	0.995 (0.992, 0.999)
Maternal Diabetes	1.40 (1.11, 1.76)
Fetal Male Gender	1.12 (1.00, 1.25)
Birthweight (continuous in grams)	1.0002 (1.0001, 1.0003)
Prior Cesarean section	1.57 (1.24, 1.99)

CONCLUSION:

The success rate of external cephalic version is 75.10%. Numerous variables were found to be significantly associated with success and failure and can guide practitioners in counseling patients as potential candidates for external cephalic version.

F-047

Neonatal Mortality among Term Deliveries: Is There a Racial Difference? Joel D Larma. *Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine, The Ohio State University, Columbus, OH, USA.*

OBJECTIVE:

To determine the relationship between maternal race and neonatal mortality in infants born between 37 and 42 weeks gestation using a large population based registry.

STUDY DESIGN:

This is a population based retrospective cohort study of all birth records using the National Health Center for Vital Statistics 2008 Natality Public Use File. The primary outcome was neonatal mortality and the primary predictor variable was maternal race, The reference group for the regression analysis was maternal white race. The results were performed independently for each week of gestation between 37 and 42 weeks. Initial univariate analysis was performed using Chi-square or Fisher's exact tests for categorical variables and the t-test or Mann-Whitney U test for continuous variables. A multiple

logistic regression model was performed using predictor variables that were found to alter the primary OR by 10-15%. Goodness of fit was assessed using the Hosmer-Lemeshow test. All analyses were performed using STATA 11.0.
RESULTS:
There were 4,145,887 births included in the final analysis with 27,968 cases of infant mortality yielding a neonatal mortality rate of 6.69 cases per 1000 births. Among deliveries between 37 and 42 weeks gestation, there were 3,007,930 births and among these there were 7219 neonatal deaths (neonatal mortality rate of 2.40 per 1000 births). The neonatal mortality rate was 3.50 per 1000 births among black women and 2.18 per 1000 births among white women. Variables found to be significant confounders included: maternal weight gain, paternal race, number of prenatal visits and marital status. The adjusted odds of neonatal mortality were found to be higher among black women relative to white women at all gestation ages (Table). The Hosmer-Lemeshow test was nonsignificant indicating goodness of fit.

OR for Neonatal Mortality by Week of Gestation Black race referent to white race	aOR (95% CI)
37 weeks	1.21 (1.05, 1.40)
38 weeks	1.12 (0.99, 1.25)
39 weeks	1.17 (1.04, 1.31)
40 weeks	1.22 (1.06, 1.39)
41 weeks	1.18 (0.90, 1.54)
42 weeks	1.88 (0.96, 3.71)

CONCLUSION:
At all gestational ages, the adjusted odds of neonatal mortality are increased among black women compared to white women even after controlling for confounding variables.

F-048

A Population Based Analysis of Racial Disparity in Late Preterm Birth. Joel D Larma. *Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine, The Ohio State University, Columbus, OH, USA.*
OBJECTIVE:
To determine the association between late preterm birth and maternal race using a large population based birth registry.
STUDY DESIGN:
This is a population based retrospective cohort study of all birth records using the National Health Center for Vital Statistics 2008 Natality Public Use File. The primary outcome was delivery between 34 and 0/7 to 36 and 6/7 weeks gestational age and the primary predictor was maternal race. The referent group for the regression analysis was white race. Initial univariate analysis was performed using Chi-square or Fisher's exact tests for categorical variables and the t-test or Mann-Whitney U for continuous variables. A multiple logistic regression model was performed using predictor variables that were either chosen a priori or were found to alter the primary OR by 10-15%. Goodness of fit was assessed using the Hosmer-Lemeshow test. All analyses were performed using STATA 11.0.
RESULTS:
There were 4,255,156 births included in the final analysis. The following variables were found to be significantly associated with late preterm birth and were included as covariates in the final regression model: maternal age, marital status, paternal age, number of prenatal visits, maternal weight gain, gender of fetus and prepregnancy weight gain. The adjusted odds ratio for late preterm birth for black parturients relative to white parturients was 1.16 (95% CI 1.13, 1.20). The Hosmer-Lemeshow test was nonsignificant indicating goodness of fit.

OR for Late Preterm Birth	aOR (95% CI)
Black Race	1.16 (1.13, 1.20)
Maternal age (continuous in years)	1.011 (1.009, 1.013)
Unmarried	1.06 (1.05, 1.08)
Paternal age (continuous in years)	0.998 (0.996, 0.999)
Number of Prenatal Visits (continuous)	0.943 (0.942, 0.945)
Marternal Weight gain (continuous in pounds)	0.994 (0.993, 0.995)
Male gender of fetus	1.09 (1.08, 1.100)
Prepregnancy Weight (continuous in pounds)	0.998 (0.9981, 0.9984)

CONCLUSION:
There is a significantly increased odds of late preterm birth among black mothers relative to white mothers even after controlling for potential confounding variables.

F-049

Fetal Mortality: Is Prenatal Care Preventive? Joel D Larma. *Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine, The Ohio State University, Columbus, OH, USA.*
OBJECTIVE:
To determine the relationship between the number of prenatal visits and fetal mortality using a large population based birth and fetal death registry.
STUDY DESIGN:
This is a population based retrospective cohort study of all births between 20 and 42 weeks gestation using the National Health Center for Vital Statistics 2005 Fetal Death Public Use File Data Set. The primary outcome was fetal demise and the primary predictor was number of prenatal visits, which was treated as a continuous variable. The referent group for the regression analysis was live births from the 2005 Natality Public Use File. Initial univariate analysis was performed using Chi-square or Fisher's exact tests for categorical variables and the t-test or Mann-Whitney U for continuous variables. A multiple logistic regression model was performed using predictor variables that were either chosen a priori or were found to alter the primary odds ratio by 10-15%. Goodness of fit was assessed using the Hosmer-Lemeshow test. All analyses were performed using STATA 11.0.
RESULTS:
Among births that occurred at 20 weeks gestational age and beyond, there were 25,931 fetal deaths noted. The number of live births in 2005 was 4,145,619 with a corresponding fetal morality rate of 6.22 cases per 1000 births. The following variables were found to be significantly associated with fetal mortality or were determined a priori and were included in the final model: race, marital status, maternal weight gain, tobacco use, maternal hypertension, fetal gender and congenital anomalies. The adjusted odds ratio (aOR) for fetal mortality associated with one prenatal visit was 0.95 (95% CI 0.94, 0.96). The Hosmer-Lemeshow test was nonsignificant indicating goodness of fit.

OR for Fetal Mortality	aOR (95% CI)
Number of Prenatal Visits (continuous)	0.95 (0.94, 0.96)
Black Race	0.86 (0.73, 1.01)
Unmarried	1.07 (0.99, 1.15)
Maternal weight gain (continuous)	0.99 (0.986, 0.992)
Tobacco use	1.38 (1.26, 1.52)
Maternal hypertension	0.94 (0.84, 1.04)
Male gender	1.08 (1.02, 1.15)
Congenital anomalies	5.79 (5.28, 6.35)

CONCLUSION:
For every one prenatal visit attended, the odds of fetal mortality are decreased by 5%, even after controlling for potentially confounding variables.

F-050

Increased Neonatal Body Fat Percentage Is Associated with an Increased Risk of Caesarean Section. Fergus P McCarthy,[1] Ali S Khashan,[1] Mairead Kiely,[2] Deirdre M Murray,[3] Jonathan Hourihan,[3] Dharmintra Pasupathy,[4] Louise C Kenny.[1] *[1]The Anu Research Centre, University College Cork, Cork, Ireland; [2]School of Food and Nutritional Sciences, University College Cork, Cork, Ireland; [3]Department of Paediatrics and Child Health, University College Cork, Cork, Ireland; [4]Division of Women's Health, King's College London, London, United Kingdom.*
Objective: In the United States, over one million Caesarean sections are performed annually with Caesarean sections accounting for over thirty per cent of births in 2008. Large for gestational age (LGA) infants as defined by customized percentiles greater than the 90th centile are at increased risk of being delivered by Caesarean section. The contribution of high neonatal body fat percentage (%BF) to this increased risk remains unclear. We hypothesized that high %BF has a stronger association with delivery by Caesarean section than LGA.
Methods: As part of the BASELINE birth cohort study (www.baselinestudy.net), neonatal %BF was measured within the first 4 days of life using air-displacement plethsymography (n=1240). A binary variable was created to indicate whether neonatal %BF was above the 90th percentile. Logistic regression was used to examine the association between neonatal %BF above

Friday

the 90th percentile and mode of delivery and LGA and mode of delivery. All analyses were adjusted for infant gender; maternal BMI, smoking and alcohol; and maternal and paternal %BF determined 4 site skin fold.
Results: LGA infants had increased %BF compared to normally grown infants (mean difference 4.4%; 95% C.I. 3.7, 5.1). Infants with %BF above the 90th percentile had significantly increased risk of Caesarean section (OR=2.3, [95% CI: 1.42-3.77]). This association was slightly stronger than the association between LGA infants and delivery by Caesarean section (OR=2.06, [95% CI: 1.29-3.29]).
Conclusion: Babies with increased %BF and babies that are LGA have a higher risk of delivery by Caesarean section. The association between %BF and delivery by Caesarean section is slightly stronger than the association between LGA infants and delivery by Caesarean section. %BF may therefore more closely reflect the macrocosmic infant. Ongoing work is investigating the association between %BF and other adverse pregnancy outcomes.

F-051

Immigrant Status and Racial Disparities in Early Exclusive Breastfeeding in Massachusetts. Linda M O Keeffe,[1] Emily Lu,[2] Ali Khashan,[3] Patricia M Kearney,[4] Renata Roney,[5] Hafsatou Diop.[2] *[1]National Perinatal Epidemiology Centre, University College Cork, Ireland; [2]Bureau of Family Health and Nutrition, Massachussets Department of Public Health, USA; [3]Anu Research Centre, University College Cork, Ireland; [4]Department of Epidemiology and Public Health, University College Cork, Ireland; [5]Mailman School of Public Health, Columbia University, NY, USA.*
Background: Research suggests that immigrants have higher breastfeeding rates than the native United States population and that immigrant women within racial groups also have higher breastfeeding rates than native women of the same race. However, more research on the role of immigrant status in breastfeeding rates within racial groups is required to investigate these suggested associations further.
Methods: Using Massachusetts Pregnancy Risk Assessment Monitoring System (PRAMS) data, a weighted survey representative of 221,014 live-births, we conducted a retrospective cohort study on the association between race and immigrant status and exclusive breastfeeding at 4 weeks postpartum. We used log-linear binomial regression controlling for maternal age, education, pre-pregnancy body mass index, parity, enrolment in the Women, Infant and Children Nutrition Program, gestational diabetes, method of delivery, and race and immigrant status interactions. Maternal immigrant status was defined as being US or Non-US born. We defined exclusive breastfeeding at 4 weeks as feeding a baby with breast milk only, up to 4 weeks postpartum.
Results: Overall, we did not find any evidence that immigrant women were more likely to breastfeed exclusively at 4 weeks{adjusted relative risk, [aRR] = 1.11, 95% Confidence Interval, [95% CI] 0.89-1.38}. We also did not find any evidence within each race category that immigrant women were more likely to breastfeed exclusively at 4 weeks compared to their native counterparts. However, Black women {[aRR] =1.30, 95% CI, 1.10-1.52} were more likely to breastfeed exclusively compared to white women at 4 weeks.
Conclusion: Our findings suggest that immigrant status does not impact overall exclusive breastfeeding at 4 weeks or exclusive breastfeeding in individual racial groups as has previously been reported in the literature. Further research is required to investigate the higher exclusive breastfeeding rate detected in Black women which conflicts with evidence that suggests that Black women have the lowest breastfeeding rates in the United States.

F-052

The Association between Gestational Diabetes Mellitus and Exclusive Breastfeeding in Early Postpartum in Massachusetts. Linda M O Keeffe,[1] Emily Lu,[2] Ali Khashan,[3] Patricia M Kearney,[4] Renata Roney,[5] Hafsatou Diop.[2] *[1]National Perinatal Epidemiology Centre, University College Cork, Ireland; [2]Bureau of Family Health and Nutrition, Massachussets Department of Public Health, USA; [3]Anu Research Centre, University College Cork, Ireland; [4]Department of Epidemiology and Public Health, University College Cork, Ireland; [5]Mailman School of Public Health, Columbia University, NY, USA.*
Background: The Agency for Healthcare Research and Quality guidelines promote exclusive breastfeeding in the general population from birth up to 6 months postpartum. Current research also suggests that exclusive breastfeeding among women with gestational diabetes mellitus (GDM) could potentially reduce the risk of developing type 1 and 2 diabetes for both women and their offspring. Understanding breastfeeding patterns among women with GDM is essential for improving the short and long-term health of both mother and child.
Objective: To compare the prevalence of exclusive breastfeeding among women with and without GDM by conducting a retrospective cohort study examining the association between GDM status and exclusive breastfeeding at 4 and 8 weeks postpartum.
Methods: Using Massachusetts Pregnancy Risk Assessment Monitoring System (PRAMS) data, a weighted survey representative of 221,014 live-births, we conducted a log-linear binomial regression controlling for maternal race, age at delivery, education, pre-pregnancy body mass index, parity, enrolment in the Women, Infant and Children Nutritional Program, smoking status and method of delivery. We defined exclusive breastfeeding at 4 or 8 weeks as feeding a baby with breast milk only, up to 4 or 8 weeks postpartum.
Results: Overall, 6.6% of women had GDM. At 4 weeks, 26.9% of these women were exclusively breastfeeding compared to 39.5% of women who did not have GDM. At 8 weeks 24.8% of women with GDM breastfed exclusively compared to 34.1% of women who did not have GDM. Women with GDM were less likely to breastfeed exclusively at 4 weeks {adjusted relative risk [aRR] = 0.71, 95% Confidence Interval [95%CI] 0.56-0.91} and 8 weeks postpartum [aRR= 0.76, 95% CI, 0.59-0.99].
Conclusion: Our findings showed lower rates of exclusive breastfeeding among women with GDM compared to women without GDM and indicate a requirement for intensified efforts in both policy and practice to increase exclusive breastfeeding rates in women with GDM.

F-053

Increased Risk of Large for Gestational Age Births in Women of Advanced Maternal Age. Sinead M O'Neill,[1] Louise C Kenny,[2] Tina Lavender,[3] Roseanne MacNamee,[4] Tracey Mills,[3] Ali S Khashan.[2] *[1]NPEC, University College Cork; [2]Anu Research Center, University College Cork; [3]Maternal and Fetal Health Research Center, University of Manchester; [4]Biostatistics Group, University of Manchester.*
Background:Delayed pregnancy is a growing trend in many countries.In the USA, the proportion of first births to women over 35 years increased eight fold between 1970-2006.In the UK between 1989-2009,the number of livebirths to women over 40 almost trebled[1].Current evidence suggests a strong association with increased risk of adverse pregnancy complications,including small-for-gestational age (SGA) and large-for-gestational age (LGA).
Methods:We examined the effect of advanced maternal age on the risk of SGA (birthweight <5th percentile) and LGA (birthweight>95th percentile) using data from the North Western Perinatal Survey[2],which included information on all singleton livebirths in the region between 2004-2007.Maternal age was divided into 2-year age categories.We compared pregnancy outcomes in women of advanced age (30+) with a younger reference category (20-29 years).Women aged <20 were excluded.Logistic regression analyses with adjustment for infant sex,parity,ethnicity,social deprivation and BMI were used.Sensitivity analyses were performed to explore the effect of parity and social deprivation on the results.
Results:The study cohort consisted of 213,817 births. 122,849 (57%) of the population studied were white,with older mothers having lower levels of social deprivation.The OR estimates supported an association between advanced maternal age and risk of LGA but not SGA (Figure 1).Parity did not influence the results while the most socially deprived women appeared to have a slightly higher LGA risk.**[Figure 1]**

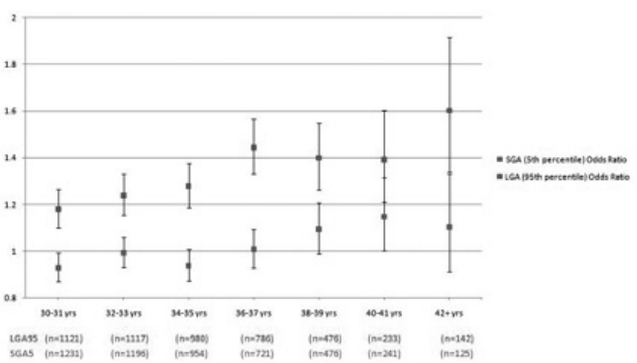

Figure 1: Risk of SGA (5th percentile) and LGA (95th percentile) Delivery According to Increasing Maternal Age

Conclusions: In this population later maternity is associated with an increased risk of LGA but not SGA. The risk is independent of parity and remains after adjusting for the confounding effects of social deprivation.The reasons underlying this trend are complex and further research is warranted.

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F-054

Transgenerational Effects of Prenatal Dutch Famine Exposure on Grand-Offspring Body Composition. Rebecca C Painter,[1] Marjolein V Veenendaal,[2] Peter D Gluckman,[3] Mark A Hanson,[4] Tessa J Roseboom.[2] *[1]Gynaecology and Obstetrics, Medical Centre Alkmaar, Alkmaar, Netherlands; [2]Clinical Epidemiology and Biostatistics, Academic Medical Centre, Amsterdam, Netherlands; [3]Liggins Institute, University of Auckland, Auckland, New Zealand; [4]Developmental Origins of Adult Disease Centre, University of Southampton, Southampton, United Kingdom.*

Objective Maternal undernutrition (F0) during gestation is associated with increased metabolic and cardiovascular disease in the offspring (F1). We previously demonstrated increased neonatal adiposity in grand-offspring (F2), but only among F2s of F1 mothers. We investigated whether famine exposure in utero leads to altered body composition in adulthood in the grand-offspring.

Population The offspring of a cohort (n=2414) of men and women born in the Wilhelmina Gasthuis in Amsterdam between November 1943 and February 1947.

Methods We approached the grand-offspring (F2) through their parents, who were 1371 eligible cohort members (F1) born around the time of the 1944–45 Dutch famine, who had been exposed or unexposed to famine in utero. Participating F2s completed an online questionnaire.

Main outcome measures Self-reported adult length, weight and body mass index of grand-offspring (F2).

Results Among 360 (225 women, 135 men) participating F2s, paternal F1 famine exposure in utero was associated with increased F2 adult BMI (+1.6 kg/m2, p=0.006) and weight (+4.1 kg, p=0.04), compared to F2 whose parents had not been exposed to famine in utero. The association remained unaltered after adjusting for possible confounders, including F2 age, F2 sex, and F2 birth weight. We did not observe any effects on F2 body composition after maternal in utero famine exposure.

Conclusions Prenatal F1 famine exposure is associated with increased F2 adult BMI and weight, but only among the offspring of F1 men who had been exposed to famine in utero. Our findings imply that the effects of famine exposure in utero are not limited to the F1 generation but persist in the F2 generation, also through the paternal line.

F-055

Social Deprivation and Adverse Perinatal Outcomes among Western and Non-Western Pregnant Women. Jashvant Poeran,[1] Arno FG Maas,[1] Erwin Birnie,[2] Semiha Denktas,[1] Eric AP Steegers,[1] Gouke J Bonsel.[1,3,4] *[1]Obstetrics and Gynaecology, Erasmus MC, Rotterdam, Netherlands; [2]Institute of Health Policy and Management, Erasmus University, Rotterdam, Netherlands; [3]Rotterdam Midwifery Academy, Netherlands; [4]Public Health, Erasmus MC, Rotterdam, Netherlands.*

BACKGROUND. In the city of Rotterdam adverse perinatal outcome rates exceed the Dutch average. Social deprivation is thought to play a crucial role. As of 2008, a combined variable indicating a neighbourhood's socioeconomic status (SES), 'Social Index' (SI), is created annually for the city of Rotterdam.
OBJECTIVE. To study the effect of a combined social deprivation variable on adverse perinatal outcomes among Western and non-Western pregnant women.
METHODS. Main determinant was the 2008/2009 mean SI, as a proxy for the neighbourhood socioeconomic status, continuous as well as grouped in 5 categories. Data on perinatal outcomes were obtained from The Netherlands Perinatal Registry for the city of Rotterdam (2000-2007, n=56,443 single pregnancies). Adverse perinatal outcomes were perinatal mortality, congenital abnormalities, preterm birth, intrauterine growth restriction (IUGR) and low Apgar score. Simple crosstabs were created making use of prevalences of adverse perinatal outcomes for each SI category, after which tests for trends and multilevel regression analysis were used to determine an actual effect.
RESULTS. The strongest effects were seen when the lowest SI category (low SES) was compared with the highest SI category (high SES), for Western women. For increasing SI categories, a decreasing trend was seen for Western women for rates of spontaneous and iatrogenic preterm birth (57.2-34.1 and 35.2-19.0 per 1000, respectively), IUGR (119.6-59.4 per 1000), low Apgar score (10.9-8.2 per 1000) and perinatal mortality (14.9-7.6 per 1000). This is also verified by highly significant p-values (<0.001) when testing for trend. Less clear or no trends were seen for non-Western women. These strong effects for Western women were confirmed by significant odds ratios estimated from multilevel regression analysis.
CONCLUSION. Social deprivation exhibits adverse effects on perinatal outcomes in the large multi-ethnic city of Rotterdam. These negative effects are larger among Western women as compared to non-Western women. This finding possibly identifies a group which would have a lot to benefit from specific health promoting programs in combination with targeted social welfare, than previously thought.

F-056

A New Screen-and-Advice Model for Psychopathology and Psychosocial Problems in Urban Pregnant Women: Psychometric Proporties and Pregnancy Outcomes. Chantal Quispel,[1,2] Gouke J Bonsel,[1,3] Tom AJ Schneider,[1] Mijke P Lambregtse-van den Berg.[2] *[1]Obstetrics and Gynecology, Erasmus Medical Centre, Rotterdam, Netherlands; [2]Psychiatry, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Public Health, Erasmus Medical Centre, Rotterdam, Netherlands.*

Background
Urban areas show increased adverse pregnancy outcomes related to psychiatric and psychosocial problems during pregnancy. We developed a Personal Digital Assistant (PDA)-based self-report screening model which includes tailored intervention advices. We tested the model after adaptation to local care pathways.
Methods
Follow-up study among 3 unselected cohorts of pregnant women (n=621), who booked at Erasmus Medical Centre and 2 midwifery practices in Rotterdam. Women completed the PDA-tool while waiting. Suggested interventions (screen-output) were discussed subsequently. Psychometric and diagnostic performances of the model were established. Pregnancy outcomes were obtained postpartum, including maternal complications, preterm birth, SGA and mode of delivery.
Results
Response rate was 94%. Internal reliability ranged 0.88-0.90, test-retest reliability ranged 0.64-1.00. Positive predictive value was 86% and negative predictive value was 97%. No interpractice psychometrical differences were observed. Migrant women received more often an intervention advice than native women (p<0.001).
Preliminary analyses of 382 cases showed an 83 gram difference in birth weight in detriment of women with EPDS scores of 12 or more, compared to women with EPDS scores below 13 (not significant). Full analysis will be completed next month.
Conclusion
The feasibility of this integral model appeared good and psychometric properties of our screen-and-advice tool were favorable under routine conditions. The technical flexibility renders the model suitable for broader application. Local care pathways can easily be incorporated into the model. We suggest implementation in prenatal care in urbanized settings, to make tailored mental health care broadly available.

F-057

Correlates of Unplanned Pregnancy among Low-Income Women. Mahbubur Rahman, Abbey B Berenson. *Center for Interdisciplinary Research in Women's Health and Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Background: Unplanned pregnancies may have a negative impact on both maternal and child health as well as the relationship of the parents. This study aimed to identify correlates of unplanned pregnancies among low-income women attending public clinics in Southeast Texas.
Methods: We conducted this study based on cross-sectional data gathered from 2270 females (16-40 years old) attending three publicly funded reproductive clinics in Southeast Texas between July 2010 and August 2011. Data were collected through self administered questionnaires. History of prior unplanned pregnancies, demographics, life-style variables, sexual behaviors as well as scores on the Beck Depression Inventory (BDI) and stress scales were assessed. Analyses of contingency tables were performed to identify significant bivariate associations. Correlates achieving bivariate significance at $P<.2$ were entered into a logistic regression model to confirm the bivariate associations.
Results: In total, 47.8% of women reported an unplanned pregnancy at least once in their lifetime. Multivariate logistic regression analysis showed an association between a history of an unplanned pregnancy and number of prior pregnancies (adjusted odd ratio [AOR]=1.71; 95% confidence interval [CI],

1.58-1.85, *P*<.001), ≥2 lifetime sexual partners (AOR=1.84; 95% CI, 1.35-2.52, *P*<.001), smoking (AOR=1.47; 95% CI, 1.14-1.88, *P*=.002), and BDI score (AOR=1.04; 95% CI, 1.01-1.08, *P*=.024). A variable assessing woman's attitude toward unprotected sex ("unprotected sex is exciting") also showed statistical significance (AOR=1.57; 95% CI, 1.26-1.96, *P*<.001). Stress scores and age at first sexual intercourse did not achieve statistical significance in the multivariate logistic regression model although they were significant on bivariate analysis. Conclusions: Findings of this study may help in the design of programs intended to decrease unplanned pregnancies.

F-058

Development of a Maternal Fetal Tissue Bank: A Useful Investment in Maternal and Fetal Health Research. Donna A Santillan,[1] Kimberly K Leslie,[1] Wendy S Hamilton,[1] Eileen M Sweezer,[1] Ryan B Empey,[1] Eric Tyler,[2] Jona M Rushing,[1] Barbara J Stegmann,[1] Stephen K Hunter,[1] Mark K Santillan.[1] *[1]Obstetrics & Gynecology, University of Iowa Hospitals & Clinics, Iowa City, IA, USA; [2]Carver College of Medicine, University of Iowa, Iowa City, IA, USA.*

Objective: The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Scientific Vision Workshop on Reproduction identified ample tissue availability with the corresponding clinical information as a major challenge for the performance of translational research. Longitudinal pregnancy biosamples would be most beneficial to research. Therefore, our objective was to create a pregnancy focused biorepository and clinical data warehouse to facilitate maternal and fetal health research.

Methods: IRB approval was obtained for a Maternal Fetal Tissue Bank (MFTB) to collect maternal and fetal samples linked to numerous clinical variables. Eligible obstetric patients from the University of Iowa Hospitals & Clinics were approached for participation. After consenting, each time a medically indicated sample of blood, urine, or amniotic fluid is taken, a sample is also banked. Cord blood and placental tissue are stored. Clinical data is automatically abstracted from the electronic medical record; 20% are validated by hand to confirm accuracy.

Results: In the first 17 months, we enrolled 607 women and their fetuses into the MFTB, with a 72.7% recruitment rate. 175 women have also agreed to participate in our registry for future studies. Of the 407 delivered women, 395 (97.1%) delivered at the University of Iowa Hospitals & Clinics. We collected 277 cord blood samples (70.1%), 86 placentae (21.8%), 4 amniotic fluid samples, 152 maternal urine samples, and 881 maternal blood samples. Data elements such as demographic variables, obstetric history, body mass index, gestational age at delivery, and birthweight have been verified.

Conclusions: Nearly 3/4 of women have been receptive to participating in the MFTB which provides no direct benefit to them. No participants have withdrawn from the study. Currently, samples and clinical data from our repository are being utilized in 7 studies. The collected samples have resulted in publication and grant submissions and are actively being used by our residents, fellows, and faculty for research. Our unique method of sample collection and clinical data acquisition allows for rapid assessment of important questions in maternal and fetal health.

F-059

Universal Low Knowledge on Preconception Folic-Acid Use in a Multi-Ethnic Urban Population. S Temel,[1] O Erdem,[2] AJJ Voorham,[2,3] GJ Bonsel,[1,3,4] EAP Steegers,[1] S Denktas.[1] *[1]Obstetrics & Gynaecology, Erasmus MC, Netherlands; [2]Research, Municipal Health Service Rotterdam-Rijnmond, Netherlands; [3]Research, Rotterdam Midwifery Academy, Netherlands; [4]Public Health, Erasmus MC, Netherlands.*

Background

Despite campaigns to promote folic-acid (FA) supplementation the number of women who take FA supplements in the recommended period remained low over the years (37% in the Netherlands). In this study we aimed to determine factors associated with (in)adequate knowledge of men and women on preconceptional FA supplementation in a large multi-ethnic city in the Netherlands.

Methods

Data were obtained from the Annual City Survey of Rotterdam (2007, 2009, 2010). Respondents were asked whether a woman who wishes to become pregnant should take FA supplementation before she tries to become pregnant. Descriptive and multiple multinomial logistic analyses were performed.

Results

Over 2007-2009, adequate knowledge of preconceptional FA supplementation has significantly changed from 29.9% to 35.8%. No further increase was observed in 2010. Significantly associated determinants with inadequate preconceptional FA knowledge were: gender, age, ethnicity, educational level, employment status, and children in household.

A woman who wishes to become pregnant, should take FA supplementation before she tries to become pregnant with 'yes, she certainly should' as reference category (n= 4215)

	No, that's not necessary	Don't know/ not relevant
	OR (95% CI)	OR (95% CI)
Gender (ref: women)		
Men	1.73 (1.38 to 2.16)**	3.21 (2.63 to 3.92)**
Age in years (ref: 25-44)		
16-24	3.94 (2.79 to 5.55)**	4.27 (3.11 to 5.86)**
45-64	2.59 (2.04 to 3.30)**	2.42 (1.95 to 3.00)**
65-75	2.62 (0.95 to 7.23)	1.96 (0.75 to 5.08)
Ethnicity (ref: Dutch)		
Immigrants	2.68 (2.02 to 3.55)**	2.20 (1.69 to 2.86)**
Europeans, other Western/non-Western	1.56 (1.08 to 2.25)*	1.30 (0.92 to 1.83)
Educational level (ref: high)		
Vey low	1.39 (0.85 to 2.26)	2.04 (1.30 to 3.20)*
Low	1.48 (1.10 to 1.99)*	1.85 (1.41 to 2.43)**
Moderate	1.06 (0.82 to 1.38)	1.33 (1.05 to 1.68)*
Employment status (ref: employed)		
Unemployed	1.63 (1.19 to 2.23)*	1.27 (0.93 to 1.72
Children in household (ref: yes)		
No	1.55 (1.24 to 1.94)**	2.32 (1.90 to 2.84)**
Religiousness (ref: yes)		
No	1.10 (0.88 to 1.38)	0.92 (0.75 to 1.13)

P-value: * <0.05; ** <0.001

Conclusion

Adequate knowledge on preconceptional FA supplementation is still too low. Interventions to increase overall awareness among the general population and especially among high risk groups are urgently needed.

F-060

Determinants of Preconception Care Attendance. Lessons from a Multi-Ethnic Cross-Sectional Survey in an Urban Population. Sevilay Temel,[1] Erwin Birnie,[1,2] Toon AJJ Voorham,[3] Bonsel J Gouke,[1,3,4] Eric AP Steegers,[1] Semiha Denktas.[1] *[1]Obstetrics & Gynaecology, Erasmus MC, Netherlands; [2]Health Policy and Management, Erasmus University Rotterdam, Netherlands; [3]Research, Rotterdam Midwifery Academy, Netherlands; [4]Public Health, Erasmus MC, Netherlands.*

Background

Evidence accumulates on the periconceptional period as the critical stage of exposure to perinatal risks. Despite significant prospective benefits of preconception care (PC), use of consultations is lower than expected. We explore likely predictors of uptake of PC in a population based study using a health behaviour model, the so-called Attitude–Social Influence–Self Efficacy –or ASE-model.

Methods

The study was conducted in Rotterdam, the Netherlands, where perinatal mortality rates range from 2 to 37 per 1000 newborns. Ethnic background and age-stratified samples were taken from the municipal population register. A total of 3225 women, aged 15-60 years, were approached through a postal survey, with a reminder after 2 weeks. As a supportive measure, trained interviewers contacted non-responders for oral survey at home. The study population consisted of 631 women aged 15-60 years: 133 Dutch, 157 Turkish and Moroccan, 341 Surinamese and Antillean. Determinants of intention to attend PC (1-10 score) were evaluated by multiple linear regression analysis.

Results

Knowledge of the adverse effect of smoking (52% correct answers;p=0.04) and overweight/underweight (55% correct answers;p=0.003) on fertility was low. Intention to attend PC was significantly higher among immigrants (Turkish and Moroccan β 0.95; Surinamese and Antillean β 0.92 compared to native Dutch), in case of higher maternal age (β 0.03 per year), and a positive attitude towards PC (β 0.54). No relationship (β -0.89), multiparity with previous adverse perinatal outcome (β -1.36), moderate (β -1.37) and high (-1.68) educational level, having paid work (β -0.69) and perceived barriers level towards PC (β -0.12) were associated with less intention to attend PC. Immigrants had a more positive attitude towards PC than Dutch (8 vs. 6;p<0.001).

Conclusion

This study is one of the first addressing social predictors of PC. Insight in these predictors that encourage or discourage attendance of PC is essential to design tailored interventions for future parents to increase attendance.

F-061

The Preconception Diet Influences the Chance of Ongoing Pregnancy Even after IVF/ICSI Treatment. John Twigt,[1] Mette Bolhuis,[1] Eric Steegers,[1] Fatima Hammiche,[1] Wouter van Inzen,[2] Joop Laven,[2] Regine Steegers-Theunissen.[1] *[1]Obstetrics and Gynecology, Erasmus Medical Centre; [2]Department of Reproductive Medicine, Erasmus Medical Centre.*

Background: Subfertility is an increasing problem in Western countries, including the US. Relative under nutrition, due to a misbalance of macro- and micronutrient intake, affects fertility parameters in both women and men. In this study, we investigate associations between adherence to preconception dietary recommendations of couples undergoing IVF/ICSI treatment and the chance of ongoing pregnancy thereafter.

Materials and methods: Between October 2007 and December 2010, couples planning pregnancy, visiting the outpatient clinic of the department of Obstetrics and Gynecology of the Erasmus University Medical Centre Rotterdam, were offered preconception counselling. Self-administered questionnaires on general characteristics and diet were filled out and checked during the visit. Six questions, based on the dietary recommendations of the Dutch Nutrition Centre, covered the intake of main food groups (bread, fats, vegetables, fruit, meat and fish). From the answers on these questions, we calculated the Preconception Dietary Risk Score (PDR), which provides an overall estimate of the personal habitual diet. Dietary quality decreases with an increasing PDR score. We defined ongoing pregnancy as an intra-uterine pregnancy confirmed by echo. We analyzed 172 couples receiving a first IVF/ICSI treatment within a six-month timeframe after preconception counselling. We applied univariate and logistic regression analysis on the outcomes of interest using SPSS.

Results: After adjusting for age of woman, PDR of partner, BMI and couple smoking status, an inverse association was shown between the PDR of the woman and the chance of ongoing pregnancy after IVF/ICSI treatment (β=-0.39, 95% CI=-0.75 – -0.03, p=0.03). Intuitively, a one-point decline on the PDR score associates with a 1.5 fold increased chance of ongoing pregnancy, i.e., OR 1.47 (95%CI=1.03 – 2.12).

Conclusions: Our results show that increasing adherence to Dutch dietary recommendations in women undergoing IVF/ICSI treatment increases the probability of ongoing pregnancy. This data warrants further confirmation in couples achieving a spontaneous pregnancy and in randomised controlled trials.

F-062

Expression of GLUT3 and Hypoxia-Inducible Factor-1α in Placentas of Growth Restricted Fetuses. Carla Janzen,[1] Margarida Y Lei,[1] John Cho,[1] Sanjali Kumar,[1] Bo-Chul Shin,[2] Sherin Devaskar.[2] *[1]Obstetrics and Gynecology, UCLA David Geffen School of Medicine, Los Angeles, CA, USA; [2]Pediatrics, UCLA David Geffen School of Medicine, Los Angeles, CA, USA.*

Background: Intrauterine growth restriction (IUGR) is associated with increased perinatal morbidity and mortality. Glucose, an essential nutrient for fetal growth, is transported from mother-to-fetus across the placenta. Facilitated glucose transporters are present in the placenta, including isoforms GLUT1,3, and 4. Mouse studies demonstrated that GLUT3-null mutants resulted in early pregnancy loss, while GLUT3 heterozygotes exhibited fetal growth restriction. HIF-1α, a subunit of the transcription factor-inducible factor-1 (HIF-1), is a key regulator of protein expression in response to hypoxia. In addition, increased expression of HIF-1α leads to IUGR in mice.

Objective: To determine if GLUT1,3, and 4 expression changes in human placenta affected with IUGR and whether expression of HIF-1α and/or changes in DNA methylation of the GLUT3 gene are correlated to GLUT3 expression.

Methods: Placentas collected at UCLA L&D (Control n = 14; IUGR n = 7) were dissected into the maternal and fetal regions, then flash frozen. **Real-time PCR** was performed using the TaqMan probe for GLUT1,3,4. **Western Blots:** Nuclear and cytosolic fractions were extracted from the tissues, and samples were run on a SDS-PAGE gel, then transferred to nitrocellulose for antibody incubation. **Pyrosequencing:** Genomic DNA was extracted from tissues and pyrosequencing was used to analyze the methylation of CpG islands on the promoter region of the GLUT3 gene.

Results: We found a 2-fold increase in GLUT3 mRNA expression in placenta affected by IUGR as compared to control (p=0.0041, Glut3 fetal control vs IUGR). Increased GLUT3 mRNA expression in IUGR placenta correlate to increased GLUT3 protein level on the fetal aspect of the placenta by western blotting (p=0.0178, Glut3 fetal control vs IUGR). Preliminarily, 2 of the 12 CpG sites tested showed significant differences in methylation % at the GLUT3 gene. In addition, we found an increase in of HIF-1α expression in the maternal IUGR samples compared to the control samples.

Conclusions: GLUT3 expression is increased in the placenta of pregnancy affected by IUGR. Presumably, changes in GLUT3 expression are induced by hypoxic stress. Additional studies are planned to assess the mechanism of GLUT3 induction in pregnancy affected by utero-placental vascular insufficiency.

F-063

Effects of Maternal Nutrient Restriction (MNR) on Hepatocyte Nuclear Factor 4 alpha (HNF4α) Expression in Fetal Baboon Liver at Term. Cun Li,[1] Zhen-Ju Shu,[2] Thomas J McDonald,[1] Peter W Nathanielsz,[1] Mark J Nijland,[1] Laura A Cox,[3] Amrita Kamat.[2] *[1]Center for Pregnancy and Newborn Research, UTHSCSA; [2]Veterans Administration, UTHSCSA; [3]Genetics, Texas Biomedical Research Institute.*

Maternal nutrient restriction (MNR) in pregnancy elevates circulating glucocorticoids (GC) serving as a model to understand GC dependent mechanisms mediating long lasting effects of fetal environmental exposures on emergence of adult diseases like type 2 diabetes (T2D). MNR (mothers eat 70% global *ad libitum* diet of control (CON) mothers) increases fetal baboon GC and hepatic expression of PEPCK (phosphoenolpyruvate carboxykinase), the GC rate-limiting gluconeogenic enzyme, at 0.9 gestation (G). PEPCK expression in fetal liver is regulated by HNF4α, a liver-enriched nuclear transcription factor.

HYPOTHESES: 1. *in vivo* GC exposure increases fetal HNF4α levels in MNR; 2. increase in hepatic HNF4α in MNR fetuses is due to GC induced HNF4α expression.

METHODS: we performed quantitative RT-PCR (qPCR) , immunohistochemistry, and western blot analysis on liver tissues from CON and MNR fetuses obtained at CSection at 0.9 G. We also performed qPCR on 0.9G CON fetal hepatocytes either untreated or treated with dexamethasone (DM).

RESULTS: A 2-fold ($p<0.04$) increase in HNF4α mRNA levels and a 2.3-fold increase in HNF4α immunoreactivity ($P < 0.001$) was observed in 0.9G MNR *vs* CON fetal livers. Western blot revealed a similar 3-fold increase in HNF4α protein in MNR fetal liver. 100 nM DM for 24h. increased HNF4α mRNA levels 2-fold (p=0.02) in DM-treated hepatocytes vs. untreated cells.

CONCLUSIONS: These studies suggest an important role for HNF4α in GC-induced PEPCK expression during MNR. Studies are in progress to identify the role of the HNF4α increase and later life glucose intolerance following fetal exposure to increased GC levels both physiological and iatrogenic when given to women who threaten preterm labor. The study of fetal nonhuman primate hepatic cells has power to remove barriers to progress imposed by inability to study normal late gestation human fetal hepatic cells. In addition fetal liver and hepatic cells for culture cannot be obtained from human fetuses of pregnancies in which maternal nutrition is preciselycontrolled to produce a specific level of IUGR. Thus these data can enable translation to human outcomes and development of diagnostic, preventative and therapeutic measures. **HD 21350 UL1RR025767**

F-064

The Effect of Ionizing Radiation on Murine Fetal Growth. David Kanter,[1] Matthew B O'Brien,[1] Sushil Beriwal,[2] Michael W Epperly,[2] Joel S Greenberger,[2] Yoel Sadovsky.[1,3] *[1]Magee-Womens Research Institute; [2]Dept. of Radiation Oncology, University of Pittsburgh; [3]Dept. of Microbiology and Molecular Genetics, University of Pittsburgh.*

Introduction: Exposure to low-dose radiation is widespread, generally attributable to natural sources. However, occupational, medical, accidental or terrorist-related exposures remain a significant threat. Information on radiation injury to the feto-placental unit is scant and largely anecdotal. We previously showed the adverse effect of ionizing radiation on term primary human trophoblast cells (PHT). We furthered our investigation and tested the hypothesis that exposure to ionizing radiation during pregnancy impacts fetal growth. We also assessed the effect of JP4-039, a mitochondria-targeted, reactive oxygen species scavenger, on the effects of radiation on fetal growth.

Methods: Pregnant C57BL/6 mice were exposed to whole-body ionizing radiation (4 Gy) or sham on E13.5, and sacrificed on E17.5. A subset of mice received intraperitoneal JP4-039 (10 mg/kg) on E13.5, 10 minutes after irradiation or sham. After sacrifice, embryos and placentas were isolated. We measured the effect of radiation on embryo weight, placenta weight, and placental transcripts that characterize radiation injury in trophoblasts.

Results: Exposing pregnant mice to ionizing radiation at E13.5 led to a 23% reduction in median mouse embryo weight, and a 12% reduction in median placenta weight ($p<0.0001$ for both). Using high-throughput microarray analysis of mice placentas, followed by qPCR confirmation, we identified several transcripts that were upregulated by exposure to ionizing radiation, including *Cdkn1a, Gjb3* and *Tpbpb* ($p<0.05$ for all). Remarkably, treatment with JP4-039 abolished the adverse effect of radiation on embryo weight, as

Friday

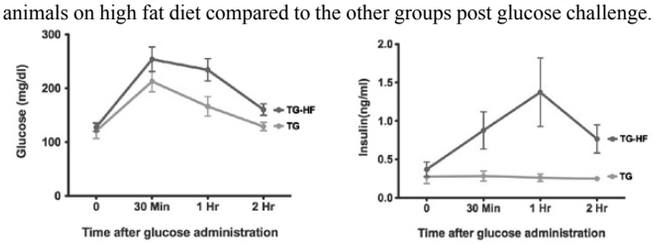
well as the changes in gene expression. Our in vivo findings confirmed those in vitro. Our time-course data showed that *CDKN1A* was significantly changed in PHTs when dose of ionizing radiation was adjusted for time after injury.
Discussion: Exposure to ionizing radiation during pregnancy reduces embryo and placenta weight, and alters the expression level of cell injury-related transcripts. These effects are mitigated by JP4-039, suggesting a targeted approach to attenuation of radiation injury during pregnancy.
This work was supported by an NIH grant U19AI068021 to JSG, with a subproject to DK and YS.

F-065

Removal of the Fetal Adrenal Medulla at 0.7 Gestation Alters Glucose-Insulin Homeostasis at 0.9 Gestation: Role of Norepinephrine in Normal Versus Growth-Restricted Fetal Sheep. Antoni R Macko, Dustin T Yates, Xiaochuan Chen, Miranda J Anderson, Amy C Kelly, Sean W Limesand. *Animal Science, The University of Arizona, Tucson, AZ, USA.*
Placental insufficiency causes intrauterine growth restriction (IUGR), and fetal hypoglycemia and hypoxemia which increase plasma norepinephrine (NE) to promote glucose sparing. The fetal adrenal medulla is the primary source of plasma NE. Our objective was to determine whether surgical ablation of the adrenal medulla at 0.7 gestation (G) alters glucose-insulin homeostasis independently of hypoxemia at 0.9 G. Sheep were randomly assigned into four groups by a combination of; control (C) or placental insufficiency-IUGR (I), and by surgical sham (S) or fetal adrenal demedullation (D) at 0.7 G. (n= 5 CS, 3 CD, 3 IS, 3 ID fetuses). At 0.9 G, each fetus underwent two studies (S1 & S2) to assess fetal metabolism. S1 in ambient normoxia, and S2 during acute fetal hypoxemia (CS & CD) or hyperoxemia (IS & ID). Placentome mass was lower in IS vs CS (119+29 v 364+20g $p<0.001$) and in ID v CD (143+12 v 302+22g, $p<0.03$). Fetal mass was lower in IS v CS (1.5+0.5 v 3.5+0.2 kg, $p<0.005$) and in ID v CD (2.1+0.3 v 3.0+0.2 kg, $p<0.05$). During basal conditions (S1): fetal plasma glucose was lower in IS v CS (10.6+1.1 v 20.2+0.9 mg/dL, $p<0.005$) and ID v CD (14.8+1.6 v 21.7+2.4 mg/dL, $p<0.05$). Arterial pO2 was lower in IS v CS (14.7+1.9 v 21.4+0.6 mmHg, $p<0.005$), and in ID v CD (12.6+1.9 v 19.8+0.7 mmHg, $p<0.01$). Fetal plasma NE was higher in IS v CS (2575.3+309.2 v 561.2+133 pg/ml, $p<0.01$) but not different between ID v CD (341.8+216.9 v 219.5+102.4 pg/ml) which were both less than IS ($p<0.05$). Insulin was lower in IS v CS (0.09+0.03 v 0.47+0.1 ng/ml, $p<0.05$), but not different between ID v CD. Acute oxygen reversal (S2): Arterial pO2 was raised to 20.4+2.9 and 22.2+2.3 mmHg in IS and ID and reduced to 10.5+0.5 and 11.9+0.5 mmHg CS and CD. Glucose was not affected by hypoxia in CS or CD. Hyperoxia increased glucose in IS to 16.2+1.3 mg/dL ($p<0.05$) but had no effect in ID. Acute hypoxia increased NE in CS (2838.9+1216.2 pg/ml, $p<0.05$) but not in CD fetuses, and reduced insulin concentrations in CS (0.22+0.06 ng/ml, $p<0.05$) but not in CD. Insulin concentrations were augmented during acute hyperoxemia in IS (0.4+0.25 ng/ml, $p<0.05$) but not in ID. Together this data supports a model to elucidate the mechanisms by which oxygen and norepinephrine regulate glucose and insulin homeostasis in normal and growth restricted fetuses.

F-066

Ecto-Nucleotide Pyrophosphate Phosphodiesterase Over-Expression in Mouse Adipocytes: A Potential Model of Gestational Diabetes. Michel Makhlouf,[1] Esther Tamayo,[1] Egle Bytautiene,[1] Phyllis Orise,[1] Huaizhi Yin,[1] Talar Kechichian,[1] George R Saade,[1] Nicola Abate.[2] *[1]Department of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA; [2]Department of Internal Medicine, The University of Texas Medical Branch, Galveston, TX, USA.*
OBJECTIVE: Gestational diabetes remains a significant source of morbidity both during pregnancy and years later through its effects on the offspring. Gestational diabetes is associated with insulin resistance and hyperglycemia. Over-expression of ecto-nucleotide pyrophosphate phosphodiesterase (ENPP1) in various tissues in transgenic models results in insulin resistance. We tested the hypothesis that mice over-expressing ENPP1 in adipocytes exhibit hyperglycemia and hyper-insulinemia following a glucose tolerance test in pregnancy.
STUDY DESIGN: Transgenic C57/Bl6 mice over-expressing ENPP1 in adipocytes (TG) and wild type C57/Bl6 mice (WT) were placed on regular chow or 60% fat diet for 6-8 weeks. TG females were then mated with WT males and WT females with TG males. On day 18 of pregnancy, an intraperitoneal glucose tolerance test was performed after a 6 hour fast. Blood glucose and serum insulin were measured at baseline and 30, 60 and 120 minutes after glucose administration. Animals were subsequently euthanized and the number of pups and their weights were determined. Statistical analysis was performed using ANOVA and post hoc multiple comparison tests as appropriate (significance: $P < 0.05$).
RESULTS: There was no difference in the number of pups per litter or in average pup weight between the groups. TG mice on regular chow had similar glucose and insulin levels post glucose challenge compared to WT controls. TG mice on a high fat diet showed significantly higher glucose levels compared to TG dams on regular chow. Serum insulin levels were 3-6 fold higher in TG animals on high fat diet compared to the other groups post glucose challenge.



CONCLUSION: Transgenic mice over-expressing ENPP1 in adipocytes exhibit features of gestational diabetes when fed a high fat diet.

F-067

Maternal Rat Low Protein (LP) Diet Results in a Switch from Proliferation to Differentiation in Offspring Pancreatic Islets. Adriana Rodriguez-Trego,[1] Maria G Ortiz-Lopez,[2] Elena Zambrano,[3] Maria Granos-Silvestre,[2] Peter W Nathanielsz,[4] Marta Menjivar.[1] *[1]Biochemistry and Physiology, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico; [2]Laboratory of Molecular Endocrinology, Hospital Juarez de Mexico, Mexico City, Mexico; [3]Instituto Nacional de Ciences Medicas y Nutricion, Salvador Zubiran, Mexico City, Mexico; [4]Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*
Maternal LP diets have been shown to impair OFF pancreatic islet development resulting in later-life β-cell failure and susceptibility to type 2 diabetes (T2D).
HYPOTHESIS: Maternal LP diet alters pancreatic islet structure and gene expression critical to β-cell differentiation and development.
METHODS: Pregnant Wistar rats ate control (C) 20% or restricted (R) 6% protein diets in pregnancy (first) and /or lactation (second letter) in four groups CC, RR, RC and CR. Litter size was adjusted to 10 pups/litter. OFF were studied at postnatal day (PND) 7. We measured male OFF β-cell mass, proliferation, and mRNA for 6 key genes in islet development, Hnf1α, Hnf4α, Pdx1, Rfx6, insulin (INS) and Glut2. Measurement: glucose by glucose oxidase method, insulin by ELISA. Beta-cell morphometric and proliferation were analyzed immunohistochemically . Beta-cell mass was obtained by multiplying β-cell fraction by pancreatic weight, The rate of proliferating β-cells was calculated with Ki67. Islets were isolated by collagenase digestion. RNA was measured by RT-PCR. Statistics: Two-way ANOVA to assess mRNAand P values < 0.05 considered significant.
RESULTS: Compared with CC, LP decreased β-cell mass and proliferation in R groups. R in pregnancy reduced islet number and size. Compared with CC - RR increased mRNA for Hnf1α, Hnf4α, and Rfx6, - RC increased Hnf1α, Pdx1, Rfx6 and INS, - CR increased Hnf1α, Hnf4α, PdX1, Rfx6 and Glut2. Insulin mRNA was highest in RC.
CONCLUSIONS: R in pregnancy and early lactation sets the trajectory for a lower growth and accelerated differentiation of pancreatic islets. Thus LP accelerates differentiation at the expense of proliferation, resulting in a smaller pancreas with potentially altered sensitivity and decreased islet reserve. These changes may result in predisposition to T2D in later-life. CONACYT (78762), and NIH HD 21350.

F-068

Does Evidence of Placental Hyperhomocysteinemia Help Explain the Relationship between Neural Tube Defects and Growth Restriction? Shayna M Norman, Methodius G Tuuli, D Michael Nelson, Anthony O Odibo, Kimberly A Roehl, Alison G Cahill. *Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA.*
Objective: Neural tube defects (NTD) are associated with growth restriction (IUGR) and abnormal folate homeostasis. We tested the hypothesis that folate deficiency, and the resulting hyperhomocysteinemia-related placental lesions occur more frequently in pregnancies with NTD, and are an etiologic link between NTD and IUGR.
Methods: We conducted a nested case-control study within a retrospective cohort study of all singleton pregnancies that delivered in a tertiary care facility from 2001-2009. Cases were defined as infants with isolated NTD

who delivered after 22 weeks. Three anatomically normal controls were selected by random-number generator, matched by year of delivery. The placental pathology results were reviewed for each case and control, blind to group membership. Placental manifestations likely linked to the vasculopathic effects of hyperhomocysteinemia were extracted from the pathology report and were defined a priori as one or more of the following histopathologies, fibrin deposition, syncytial knotting, infarct, and thrombosis. Odds ratios and 95% confidence intervals were estimated for each finding. Conditional multivariable logistic regression was used to adjust for baseline differences, grouped by exam year.

Results: Over the study period, 39 cases of women with isolated NTD were identified and compared with 117 controls. Baseline demographics of cases and controls were similar except for differences in percentages of advance maternal age (AMA) and African American race. There was no difference in individual placental pathologic lesions between cases compared to controls. Rather, there was a trend towards a lower percentage of composite lesions in controls vs. cases.

Placental lesion	NTD (n=39)	Controls (n=118)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)	P value
Fibrin Deposition	10.3%	13.6%	0.7 (0.2-2.3)	0.5 (0.1-1.6)	0.24
Syncytial Knotting	2.6%	2.5%	1.0 (0.1-9.6)	1.0 (0.1-11.7)	0.99
Infarct	2.6%	19.5%	0.1 (0.01-0.8)	0.1 (0.01-0.9)	0.04
Thrombus	12.8%	11.9%	1.1 (0.4-3.2)	1.1 (0.3-3.6)	0.88
Composite	15.4%	30.5%	0.4 (0.2-1.1)	0.4 (0.2-1.1)	0.07

* Adjusted for AMA and African American race

Conclusion: Vasculopathic effects of hyperhomocysteinemia from dysregulated folate metabolism did not occur more frequently in the placentas of women who gave birth to infants with NTDs compared to those with normal anatomy.

F-069

Growth Restriction in Dichorionic Twin Pregnancy: Investigating the Role of Inhibin A, Activin A and sEndoglin. Clare O'Loughlin, Lynne Kelly, Ifeomah Offiah, Shanti Muttukrishna, Keelin O'Donoghue. *Obstetrics and Gynaecology, Anu Research Centre, Cork University Maternity Hospital, University College Cork, Cork, Ireland.*

Fetal growth restriction (FGR) is associated with increased perinatal morbidity and mortality. Studies show between 12% and 47% of twins lie below the tenth percentile for gestation. Activin A and inhibin A are glycoproteins belonging to the transforming growth factor beta (TGF-β) superfamily of cytokines. Endoglin is a co-receptor for transforming growth factor, expressed by placental synctiotrophoblasts and endothelial cells. While the role of these proteins in pregnancy remains undefined, they are raised in singleton FGR pregnancies. We sought to determine whether these findings were applicable in twin pregnancies. Dichorionic twins present a unique opportunity to study these potential protein markers for growth restriction.

A consecutive cohort of patients with DCDA twins were recruited from between 20 and 24 weeks. Information recorded included: age; parity; body mass index; smoking status; spontaneous or assisted conception; medications taken; development of pre-eclampsia (PET);fetal growth restriction and growth discordance. Outcomes included mode of delivery, fetal morbidity and mortality, birth weights and maternal pregnancy-related complications. Twenty-three patients were recruited and all had maternal serum samples taken at 20-24 weeks, while two had second samples taken between 28 and 32 weeks. Inhibin A, activin A and sEndoglin levels were determined by ELISA. Statistical analysis was performed with SPSS and ANOVA.

Initial comparisons using Spearman's rho showed a statistically significant relationship between BMI and inhibin (p=0.035). Those diagnosed with gestational diabetes or who conceived by ART were more likely to have used medications (p=0.014; p=0.001). Univariate analysis of variance was used to determine whether there were statistically significant differences in levels of these proteins comparing normal grown controls, and those with FGR and PET. Multiple comparisons and between subject effects using the logarithmic transformation of inhibin A, activin A and sEndoglin to reduce error did not reach statistical significance. The most likely explanation for this is the low sample size. We are continuing recruitment to attain adequate power to determine whether these proteins are increased and relevant in growth restricted twin pregnancies.

F-070

Plasma Fatty Acids at Birth Predict Infant Growth. Perrie F O'Tierney,[1] Melanie Gillingham,[2] Cynthia Morris,[3] David Barker,[1] Kent L Thornburg.[1,4] *[1]Heart Research Center, Oregon Health & Science University, Portland, OR, USA; [2]Mol & Med Genetics, OHSU; [3]Public Health & Prev Med, OHSU; [4]Medicine (Cardiology), OHSU.*

Objectives: Long chain polyunsaturated fatty acids (LCPUFA) are essential for proper fetal growth, neurological and cardiovascular development. Low levels of LCPUFA are associated with increased adiposity later in life. We hypothesized that fat deposition in early infancy would be correlated with a high proportion of saturated fatty acids and low proportion of LCPUFA in plasma at birth.

Methods: Women were recruited in their third trimester as part of the Oregon Women's Study. At delivery cord blood was collected for analysis. Detailed infant body composition measurements were recorded 1 day and 6 months after delivery (Skinfolds, weight, crownheel length, head circumference). Sex-specific weight-for-length z-scores were determined using US reference data. Fatty acid profiles were quantified using gas chromatography-mass spectrometry in plasma.

Results: High infant weight-for-length z-scores at 6 months of age were associated with low neonatal LCPUFA (r= -0.48, P=0.02) and high neonatal saturated fatty acids (r=0.51, P=0.02). Similarly, the change in weight-for-length (z-scores) between birth and 6 months of age was negatively related to plasma LCPUFA at birth (r= -0.66, P=0.004) and positively related to plasma saturated fatty acids in the neonate (r=0.66, P=0.004). Neonatal body composition measures were not related to maternal or neonatal fatty acid profiles. No sex-differences were detected.

Conclusions: Low levels of LCPUFA and high levels of saturated fatty acids in neonatal plasma were associated with high infant weight-for-length at 6 months of age, but not at birth. Our findings suggest that high weight-for-length growth in early infancy is associated with low plasma LCPUFA levels at birth.

F-071

Fetal Thymic Size in Intrauterine Growth Restricted Fetuses. Elena Olearo, Pietro Gaglioti, Manuela Oberto, Giovanna Ogge, Carlotta Pace, Tullia Todros. *Department of Obstetrics and Gynecology, Univeristy of Turin, Turin, Italy.*

Introduction*:* Thymic involution is associated with atopic, autoimmune and infectious diseases in infancy and adulthood. A small fetal thymus has been observed in cases of infection, preterm delivery, chorioamnionitis and preeclampsia. Intrauterine growth restricted (IUGR) babies present thymic involution at postmortem examination but very little is known about the relationship between thymic size and causes of IUGR. ***Objective***: The aim of this study is to demonstrate that a difference in thymic size exists between small for gestational age (SGA) fetuses, likely constitutional, and IUGR secondary to placental anomalies. ***Methods:*** We studied 28 pregnancies complicated by SGA and 36 uncomplicated pregnancies. SGA was defined as fetal abdominal circumference (AC) and birth weight < 10th percentile for gestational age (GA). SGA were first divided into 2 groups, based on growth velocity and uterine artery Doppler (UAD): constitutional SGA (normal growth velocity, normal UAD) and IUGR (reduced growth velocity, abnormal UAD). IUGR were further divided using umbilical artery Doppler (uaD): IUGR with normal uaD (PI<95th percentile) and IUGR with abnormal uaD (PI≥95th percentile). Cases with chromosomal anomalies, maternal or fetal infections and multiple pregnancies were excluded. Fetal thymic volume (TV) was obtained by 3D ultrasound using Virtual Organ Computer aided Analysis (VOCAL). To exclude influence of fetal size on the dimension of the thymus, the TV/AC ratio was calculated. Student's independent T-test and one-way ANOVA test were used for comparison among groups. ***Results***: TV/AC ratio in SGA fetuses was lower than in controls *(P< 0,001)*. Among SGA, IUGR with abnormal uaD (n=10) had lower TV/AC ratio than constitutional SGA (n=9) (*P=0,01*) or IUGR with normal uaD (n=9) (*P=0,01)*. ***Conclusion***: This is one of the first studies using 3D ultrasound and VOCAL software to investigate fetal thymus. IUGR fetuses with abnormal uaD have significant reduced thymic size compared with the other subgroups, similarly as what was previously reported in preterm delivery, chorioamnionitis and preeclampsia. We speculate that thymic shrinkage in IUGR could be related to an early, subclinical infection, which compromises both trophoblastic invasion and thymic development.

F-072

A Case of Pregnancy Complicated by Adenomyosis Resulting in Severe Fetal Growth Retardation. Natsuki Ota, Takashi Yorifuji, Shintaro Makino, Taro Koshiishi, Motoi Sugimura, Satoru Takeda. *Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo, Japan.*

Introduction: In pregnancy complicated by adenomyosis, fetal growth retardation (FGR) may occur. However, few actual case reports have been described, and the mechanisms are not well unknown. We report herein a case of pregnancy complicated by adenomyosis, resulting in severe FGR. In this case, we confirmed decreased placental blood flow which was attributed to FGR with the none contrast-enhanced time-spatial labeling inversion pulse (Time-SLIP) MRA technique.

Case: The patient was a 34-year-old gravida 4, para 1 woman who had a previous fetus with FGR starting from gestational week(wks) 31. At 33wks, emergency cesarean section had been performed because of non-reassuring fetal status. The current pregnancy was a natural pregnancy. Transvaginal ultrasonography in early pregnancy showed a 7cm adenomyosis in the posterior wall of the uterine body. At 19wks, estimated fetal weight was 117 g and symmetrical FGR was identified. She was thus managed as an inpatient from 20wks. Ultrasonography showed no gross fetal abnormalities. The mother had no underlying medical disease, no infections, and no obstetric complications other than FGR. After admission, fetal well-being was frequently evaluated by ultrasonography. On admission, the umbilical diastolic blood flow was not already identified, but amniotic fluid volume was maintained. The placenta was located in the uterine fundus, away from the adenomyosis. The site of umbilical cord insertion appeared normal. At 23wks, MRI revealed the 11cm adenomyosis in the lower posterior wall of the uterus. On Time-SLIP MRA, blood flow in the left uterine artery, as the feeding artery, was toward the adenomyosis, and placental blood flow was poor. Starting from25wks, uterine artery diastolic blood flow was reversed. At 27wks, fetal movements decreased and fetal pericardial effusion and oligohydramnios were detected. Emergency cesarean section was performed. A baby girl was delivered, weighing only 292 g with an Apgar score of 1 at both 1 and 5 min. Pulmonary hemorrhage occurred at 9 days old, and the baby died. Chromosomal analysis of chorionic villi showed a normal karyotype. Histological examination of the placenta revealed no obvious cause of FGR. **Conclusion:** Time-SLIP MRA confirmed decreased placental blood flow. In this case, the cause of FGR was attributed to "vascular steal" by the uterine adenomyosis, decreasing placental blood flow.

F-073

Intertwin Discordance in Monochorionic Biamniotic Pregnancies. Francesca Pelizzoni, Francesca Russo, Maddalena Incerti, Sabrina Cozzolino, Patrizia Vergani. *Obstetrics and Gynecology, S. Gerardo Hospital.*

Objective: Intertwin birth weight discordance has been shown to be associated with an increased risk for perinatal morbidity and mortality. The aims of this studies were (1) to estimated the threshold of birth weight discordance for prediction of adverse perinatal outcome in monochorionic-biamniotic (MC) (2) to establish which sonographic growth parameter can better predict birth weight discordance.

Study design: We analyzed a cohort of MC twins pregnancies from 24 weeks gestation to the term from 1998 to 2009**.** Adverse perinatal outcome was defined as neonatal/intrauterine death or the occurrence of major morbidities. Two ecographic growth parameters were used to evaluate the intertwin discordance: (1) the estimated fetal weight (as ΔEBW, calculated as the difference of the two birthweight normalized for the bigger weight) and (2) the abdominal circumference AC (as AC ratio, calculated by division of the AC of the smaller by the AC of the larger twin, and as ΔAC, calculated as the difference between the two CA).

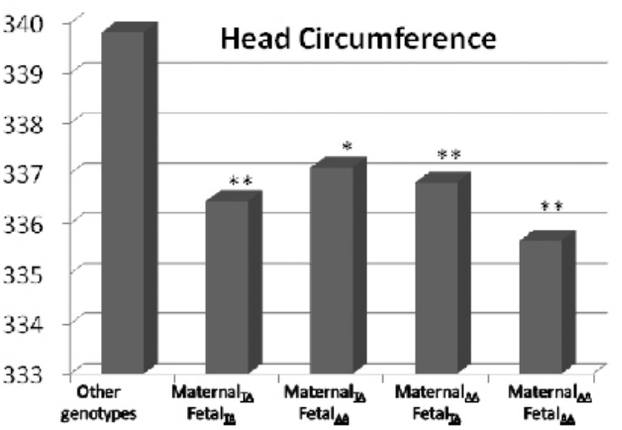
Results: A total of 221 pregancies were evaluated. Birth weight discordance was an independent predictor of adverse perinatal outcome (p=0.004). Receiver operating characteristic curve (ROC) analysis suggested that the threshold of birth weight discordance in MC twins was 15% with a sensibility (Sn) of 53% and a false positive rate (FP) of 15%; that threshold preserved a statistic significance at every gestation age. All tree parameters were good predictors of adverse perinatal outcome ($p < 0.001$). ROC curves were created for the tree sonographic parameters of discordance too. For the estimated fetal weight discrepance the ROC curve suggested a threshold of 15% with a sensibility of 47% and a false positive rate of 15%; for ΔAC the threshold was 18 mm with 53% of sensibility and 23% of false positive rate and for about the AC ratio the threshold was 0,96 with 60% of sensibility and 34% of false positive rate.

Conclusion: An intertwin birth weight discordance ≥ 15% is significantly related with adverse perinatal outcome. The most predictive sonographic parameter for weight discordance of twin is the estimated fetal weight with a threshold of 15%.

F-074

Maternal and Fetal Genotype Is Required To Understand the Full Impact of Genetics on Fetal Growth. CE Pennell,[1] JA Marsh,[1] QW Ang,[1] HR Taal,[2] LJ Palmer,[3] SJ Lye,[3] L Briollais,[3] VW Jadoe,[2] JP Newnham.[1] *[1]School of Women's and Infants' Health, University of Western Australia, Australia; [2]Department of Epidemiology, Erasmus Medical Centre, Netherlands; [3]Samuel Lunenfeld Research Institute, University of Toronto, Canada.*

Genetic variants in the fat mass & obesity-associated (FTO) gene are associated with childhood & adult obesity across multiple populations & ethnic groups. However, the effect of the maternal rs9939609 variant & the interaction between the maternal & fetal rs9939609 genotypes on fetal growth are unknown. Repeat measurements of fetal growth from ultrasound sonography from 1,377 singleton births from the Western Australian Pregnancy (Raine) Cohort were analysed using linear mixed-effects models including terms for fetal & maternal genotype & the maternal-fetal genotype interactions. Birth measurements were analysed similarly using multivariate linear regression. No associations were detected between fetal growth trajectories & either fetal or maternal rs9939609 genotypes when considered independently (without an interaction with smoking), except for femur length where the fetal A risk-allele was associated with smaller femur growth (p=0.02). Similar results were obtained for measurements of birth size. However, when maternal-fetal rs9939609 genotype combinations were considered, an increasing number of maternal A alleles was associated with smaller fetal head circumference, smaller fetal abdominal circumference, smaller fetal femur length, smaller birth weight & smaller birth length.



The heterozygote/homozygote combination of Maternal$_{AA}$-Fetal$_{TA}$ had a greater effect on fetal head circumference, birth weight & birth length than Maternal$_{TA}$-Fetal$_{AA}$, suggesting that the maternal genotype may have a greater influence on fetal growth than the fetal genotype. Replication of these results is currently underway in the Generation R cohort. The FTO mother appears to employ a nutrition-privileged process in the maintenance of maternal-fetal nutrition. For the first time we have shown that both maternal & fetal rs9939609 genotypes should be considered simultaneously to elucidate the effect on fetal growth.

F-075

Maternal Nutrition Restriction (MNR) in Non-Human Primates (NHP) Down-Regulates Mitochondrial Oxidative Phosphorylation Transcripts at 0.5G. Susana P Pereira,[1,2] Paulo J Oliveira,[1] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Center for Neurosciences and Cell Biology, Univ. Coimbra, Coimbra, Portugal; [2]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*

Malnutrition has been associated with alterations in cardiac metabolism and performance. Disruption of myocardial bioenergetics can be clinically devastating. Mitochondrial dysfunction has been related to more than 50 distinct diseases ranging from neonatal fatalities to cardiac dysfunction or neurodegeneration in the adult, and is a likely contributor to cancer and type II diabetes. In the specific case of the heart, the pre-disposition to mitochondrial bioenergetic failure can start well before birth, when signaling pathways are emerging.

The objective of the present study was to investigate the hypothesis that MNR alters the cardiac mitochondrial transcriptional profile at 0.5G.
Pregnant baboons of similar body weight were fed [italics]ad lib[/italics] (C, n=5) or, starting at 0.16 G, 70% of [italics]ad lib[/italics] fed C (MNR, n=5). Samples from the free wall of the fetal cardiac left ventricle (LV) were obtained by c-section at 0.5 G and stored at -80°C. Quantitative PCR array was used to determine mRNA expression. Data were analyzed using Graph Pad Prism v5, p-value < 0.05 considered significant.
At 0.5G several transcripts relevant to heart mitochondrial bioenergetics were decreased in the female (F) fetus, while only two transcripts were down-regulated in male (M) fetuses. Seven of the transcripts altered in F relate to mitochondrial Complex I (NDUFS2, NDUFS3, NDUFA11, NDUFA3, NDUFB2, NDUFB3, NDUFA10), one to Complex II (SDHB), one with Complex III (UQCRC1), one to Complex IV (COX4I1) and two in complex V (ATP4A, ATP6V1G3). Only two mitochondrial transcripts were different between the sexes in C fetuses, NDUFB10 and ATP5A1, and both were up-regulated in F.
MNR at 0.5 G had a marked effect on components of the mitochondrial oxidative phosphorylation apparatus, particularly in F fetuses. The data indicates that MNR promotes alterations in oxidative phosphorylation transcripts in the heart which can impact normal myocyte differentiation and cardiac development.
Supported by NIH PO1 HD023150 (MJN, PWN) and Portuguese Foundation for Science and Technology SFRH/BD/64247/2009 (SPP,PJO).

F-076

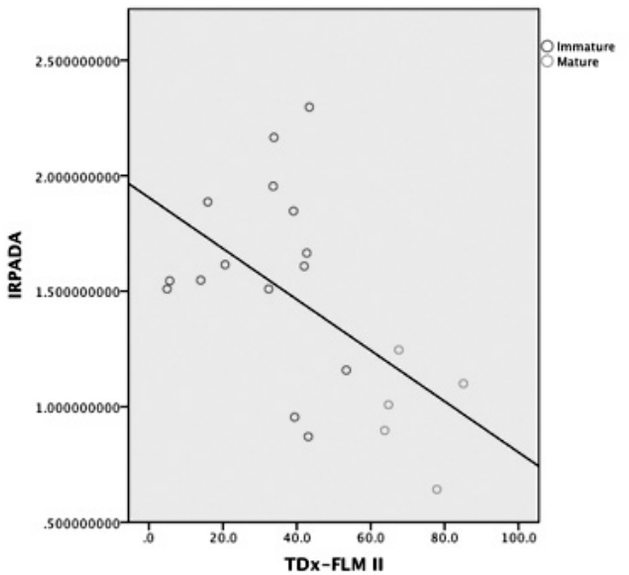
Early Pregnancy Maternal Nutrient Restriction (NR) Does Not Alter Uterine Artery (UA) Expression of Large Conductance Ca^{+2}-Dependent K^+ Channels (BK_{Ca}). H Wojciechowski,[1] X-t Liu,[1] SP Ford,[2] CR Rosenfeld.[1]
[1]Neonatal-Perinatal Medicine, UT Southwestern Medical Center, Dallas, TX; [2]Animal Sciences, Univ. of Wyoming, Laramie, WY, USA.
Background. Maternal NR during ovine placentation (28-78d gestation) causes maternal weight (Wt) loss and fetal growth restriction (FGR) at 78d. Introduction of adequate nutrients in the last half of pregnancy corrects fetal Wt by 135d. The FGR may reflect abnormal maternal and/or fetal uteroplacental blood flow (UPBF) in NR ewes and correction in late gestation. Starvation decreases UPBF >30%; UPBF during NR is unclear. BK_{Ca} modulate ovine maternal UPBF; we wondered if the FGR and subsequent accelerated fetal growth in NR ewes reflected altered UA BK_{Ca} expression. **Hypothesis.** Early pregnancy maternal NR alters UA BK_{Ca} subunit expression, contributing to FGR. **Methods.** Pregnant ewes were assigned control (C; 100% NRC recommendations) or NR (50% of NRC) diets at 28d gestation and euthanized at 78d (n=5 and 4, respectively). Additional NR ewes were changed to 100% NRC at 78d, euthanized at 135d (n=4) and compared with C, n=4. UA were collected and stored at -80°C. Samples were assayed for total/soluble protein, actin/myosin, and BK_{Ca} subunits (α, β_1, and β_2). Data are means±SD. **Results.** Maternal Wt was similar at 28d. At 78d NR maternal Wt was less than C, 64±7.5 vs. 82±3.5kg (P=0.002), as was fetal Wt, 199±18 vs. 263±29gm (P=0.005), respectively. At 78d, NR UA actin (P=0.08) and myosin (P=0.02) exceeded C 28% and 39%, respectively; soluble/total protein and BK_{Ca} composition did not differ. After enhancing NR to 100% NRC at 78d, NR maternal Wt gain at 135d was 9.8±1.7% vs. 13.7±1.3% in C (P=0.01); fetal Wt was similar (4510±644 vs. 4712±614gm, P>0.1). C UA had higher actin (50%, P=0.07) and actin/myosin ratio (56%, P=0.055); but BK_{Ca} subunit composition did not differ. Comparing values in C and NR groups at 78 and 135d, C had 11% fall in soluble protein, 43% rise in actin and 57% rise in A/M ratio (P≤0.04); BK_{Ca} subunit expression was unchanged. Realimented-NR ewes had 32% and 21% falls in soluble protein and soluble/total protein ratio (P<0.02); BK_{Ca} subunit expression was unchanged. **Conclusions.** BK_{Ca} subunit expression was similar in NR and C ewes at 78d and 135d gestation, suggesting altered BK_{Ca} expression and function may not contribute to alterations in maternal UPBF and FGR; however UPBF was not assessed. Studies of NR and maternal/fetal UPBF should be considered. (NIH HD008783-CRR and T35-DK066141-HW)

F-077

Enhanced Neonatal Thermogenesis in Cold-Challenged Lambs Following Maternal Arginine Supplementation. Michael C Satterfield, Sorin M Greff.
Animal Science, Texas A&M University, College Station, TX.
Neonatal thermoregulation in both humans and sheep is an essential physiological process mediated largely by non-shivering thermogenesis in brown adipose tissue (BAT). In humans, preterm and low-birth weight infants are highly susceptible to hypothermia; while in sheep, 40% of non-predator deaths are attributed to cold and cold-related causes. Similar to humans, BAT is responsible for 50% of the heat generated in the newborn lamb despite comprising only 2% of body weight. Previously, we found that maternal arginine supplementation increased fetal peri-renal BAT mass by 48% and 62% in lambs from undernourished and overnourished ewes, respectively. Therefore, we tested the hypothesis that increased fetal BAT will enhance neonatal thermogenesis at birth in response to cold. Multiparous Suffolk ewes (n=31) were assigned to receive either intravenous injections of L-arginine (27 mg/kg bodyweight; n=17) or sterile saline (n=14) three times daily from Day 75 to Day 125 of gestation. Following parturition, lambs were removed from their mothers, placed in a thermoneutral environment, and fed artificial colostrum per weight. At 4 h of age, lambs were cold challenged at 0°C for 2 h. Rectal temperatures were recorded at 15 min intervals. At 6 h of age all singletons and one lamb of each twin pair was sacrificed. The remaining twin lamb was challenged again at 22 h of age for an additional 2 h prior to necropsy. Rectal temperatures were higher for the duration of both cold challenges in lambs from arginine-treated ewes than lambs from control ewes (P<0.05). Interestingly, at the time of necropsy, there was no difference (P>0.10) in BAT weight between treatments. Expression of mRNAs for PGC1A, NRF1, NRF2, PPARG, B3AR, ARG2, RPS6KA1, EIF4EBP1, ODC were not affected by treatment (P>0.10) but were higher (P<0.05) at 24 than 6 h of age. There was no difference (P>0.10) in UCP1 mRNA expression. Analysis of amino acids in neonatal circulation found that serine and glycine were higher (P<0.05) in lambs from arginine-treated mothers than controls at 0, 6, and 24 h of age. Total amino acid levels decreased (P<0.05) from 0 to 6 h of age. Results indicate that maternal arginine treatment increases neonatal thermogenesis after birth. Although the underlying mechanisms remain to be elucidated, these data are a first step in improving neonatal survival and well-being both clinically and agriculturally. (Supported by USDA-NRI 2009-35206-05211)

F-078

A Non-Invasive Method To Predict Lung Maturity Using the Impedance Ratio of the Fetal Pulmonary Artery and Ductus Arteriosus. Mauro H Schenone,[1] Jacques E Samson,[1] Anju Suhag,[1] Laura Jenkins,[2] Giancarlo Mari.[1]
[1]Obstetrics and Gynecology, The University of Tennessee Health Science Center, Memphis, TN, USA; [2]Obstetrics and Gynecology, University of Tennessee Medical Group, Memphis, TN, USA.
Objective: To determine whether the measurement of the impedance ratio of the fetal pulmonary artery and ductus arteriosus (IRPADA) can accurately predict the results of fetal lung maturity (FLM) testing in amniotic fluid.
Methods: We prospectively studied pregnancies attending our ultrasound unit for clinically indicated FLM testing. An ultrasound examination including measurement of the IRPADA (ratio of the main pulmonary artery wave form's acceleration time/ejection time divided by the ratio of ductus arteriosus wave form's acceleration time/ejection time) was performed before results of the amniocentesis were known. Results of the TDx-FLM II (Abbott laboratories) and IRPADA were compared using Pearson's correlation coefficient. A receiver operator characteristic (ROC) curve was created to determine the IRPADA cut off value with optimal sensitivity and specificity. A p<0.05 was considered statistically significant.
Results: 20 consecutive measurements of IRPADA and TDx-FLM II were obtained from 16 patients. 4 patients had a second amniocentesis because of an immature result during the first amniocentesis. 5 of the TDx-FLM II measurements resulted mature (≥55), whereas the other 15 resulted immature (<55). A negative correlation was found between the IRPADA and the TDx-FLM II (r=-0.55, p=0.012). The ROC curve demonstrated that an IRPADA cut off value of 1.4 would provide a sensitivity of 100% (95%CI: 57%, 100%) and a specificity of 80% (95% CI: 55%, 93%).
Conclusion: There is an inverse correlation between the IRPADA and the TDx-FLM II; this probably reflects decreasing vascular impedance as lung maturation progresses. All cases with an IRPADA of 1.4 or higher resulted immature; therefore, Doppler ultrasound may be a useful tool in the diagnosis of FLM.



F-079

Hypothalamic Arcuate Nucleus (ARC) Neurons: Differential Effects of Leptin on Electrophysiological Properties during Postnatal Development. Xiaoping Sun, Tatsuya Fukami, Tie Li, Mina Desai, Michael G Ross. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: In mature adults, the adipocyte-derived hormone leptin acts acutely on the brain to reduce food intake by regulating the activity of hypothalamic orexigenic (neuropeptide Y; NPY) and anorexigenic (pro-opiomelanocortin; POMC) neurons, primarily in the arcuate nucleus (ARC). During the fetal and neonatal period, during which the ARC is formed, leptin has potent neurotrophic effects. However, the functional impact of leptin on specific ARC neurons during the developmental period is not clear. We investigated the effects of leptin on ARC neurons during neonatal and juvenile (weanling) age periods.

Study Design: Brain slices from postnatal rat pups (3-8, 10-13, 18-27, 28-35 days old, and adults) were used to characterize the intracellular effects of leptin on ARC neurons by whole-cell and cell-attached patch clamping techniques. Membrane potential, firing rate of action potentials and synaptic currents were recorded before and after leptin treatments.

Results: Leptin had differential impact on ARC neurons during postnatal ages. ARC neurons had the highest responsive rate to leptin (100 nM) among rats at age of 3-5 weeks. Leptin depolarized the membrane potential (6.6±1.2 mV) in 40% and hyperpolarized (-6.9±1.6 mV) in 28% of ARC neurons (n=101). In contrast, only 12% of neurons were depolarized and hyperpolarized by leptin (n=25) at age of 3-8 days, respectively. When classified by neuropeptide cell type, leptin had excitatory effects as evident by intracellular membrane *depolarization* (4.9±1.2 mV) or increased action potential firing rate in 8 of 10 POMC neurons. Conversely, leptin had inhibitory effects as indicated by intracellular membrane *hyperpolarization* (-7.1 ± 3.5 mV) or reduced firing rate in 13 out of 15 NPY neurons. Moreover, the amplitude and frequency of spontaneous inhibitory postsynaptic currents were inhibited by leptin.

Conclusion: the majority of ARC neurons are less response to acute leptin during early postnatal age. However, around weaning, the response rate increases significantly. This age dependent manner of acute leptin effects may correlate to functional development of its receptor or intracellular signaling mechanisms.

F-080

Impact of Anesthetics and Analgesics at Defined Times during Gestation on Fetal Growth in the Mouse. Larry G Thaete,[1] Stephen I Levin,[2] Andrew T Dudley.[3] *[1]Obstetrics & Gynecology, NorthShore University HealthSystem, Evanston, IL; [2]Center for Comparative Medicine, Northwestern University, Chicago, IL; [3]Molecular Biosciences, Northwestern University, Evanston, IL, USA.*

Introduction: Anesthetics and analgesics are often necessary during studies of fetal growth and development. When a study is designed to allow gestation to continue, it is important to know how various anesthetics and analgesics may affect fetal development. While preliminary observations indicated that ketamine/xylazine administered late in gestation caused 18-25% fetal growth restriction in the mouse, the influence of these agents on mouse fetal development throughout gestation is not well-characterized.

Objective: To determine the impact of several common anesthetics and analgesics, administered at defined times during pregnancy, on fetal mouse growth and development.

Methods: Wild-type, timed-pregnant C57BL/6J mice were treated on gestation day 0, 4, 6, 12, or 15 (approximating the timing of fertilization, attachment, start of organogenesis, end of organogenesis, and logarithmic growth phase) with one of three anesthetics or one of two analgesics. The anesthetics were ketamine/xylazine (100/10 mg/kg IP), isoflurane (3% for 30 min), or tribromoethanol (250 mg/kg IP). The analgesics were buprenorphine (0.1 mg/kg SC, twice/day for 2 days) or meloxicam (2 mg/kg IM, once/day for 2 days). On day 18 (term) the mice were euthanized, fetal and placental weights were recorded, and fetuses were prepared either for skeletal analysis by alizarin red staining or for general morphological analysis after Bouin's fixation. All groups were compared to an untreated control group (n=5-7 litters/group). Data are reported as mean ± SEM and were analyzed by ANOVA with $p<0.05$ representing statistical significance.

Results: Fetal growth was significantly reduced ($p<0.01$) by ketamine/xylazine administered at days 12 & 15, isoflurane at day 0, buprenorphine at days 4 & 6, and meloxicam at day 0. Skeletal analysis confirmed reduced bone growth in fetuses from dams treated with buprenorphine. Placental growth was not affected.

Conclusions: Certain anesthetics and analgesics can affect fetal growth when administered maternally. Effects by some agents given early in gestation have a lasting impact on fetal growth throughout gestation. Care must be given to the choice of anesthetics and analgesics during gestation so as to avoid significant fetal developmental consequences.

F-081

High Periconception Folate Status Affects Human Embryonic Growth. E van Uitert,[1] E Steegers,[1] A Koning,[2] J Lindemans,[3] J Laven,[1] N Exalto,[1] R Steegers-Theunissen.[1] *[1]Obstetrics & Gynecology, Erasmus MC; [2]Bioinformatics, Erasmus MC; [3]Clinical Chemistry, Erasmus MC.*

Background

Periconception folic acid supplement use and fortification has increased maternal folate status in the population and successfully reduced the occurrence of certain congenital malformations. Although folate is required for many cellular processes, effects of folate on human embryonic growth are largely unknown. Advanced ultrasound techniques provide the opportunity for very precise measurements of embryonic crown-rump length (CRL). We aim to study the influence of periconception maternal folate status on embryonic growth.

Methods

In a prospective periconception cohort study, participants enrolled between 6 to 7 weeks of pregnancy, filled out a self-reported questionnaire, and received weekly high resolution transvaginal three-dimensional ultrasound (3DUS) scans from 8 up to 13 weeks of pregnancy. To assess long-term folate status, maternal blood was collected to determine RBC folate at a median gestational age of 7^{+4} (6^{+6}-8^{+5}) weeks. Using our I-Space virtual reality system, 3DUS holograms were created to perform CRL measurements. Women with RBC folate and CRL measurements and healthy pregnancy outcome were selected for analysis. We calculated the ninetieth percentile (P90: 2122nmol/L) of RBC folate in this study population. Data were log-transformed and analyzed using a linear mixed model.

Results

A total of 390 ultrasound scans were performed in 83 women of which 370 CRLs could be measured. Regression analysis shows that CRL of embryos exposed to RBC folate above P90 is smaller, but embryonic growth rate is increased compared to the other embryos; for any 10% increase in gestational age growth increases by an extra 1.3% (p=.04). At 8^{+0} weeks of gestation, CRL is 7% (1.1mm) smaller and at 12^{+6} weeks this difference is reduced to 1% (0.6mm).

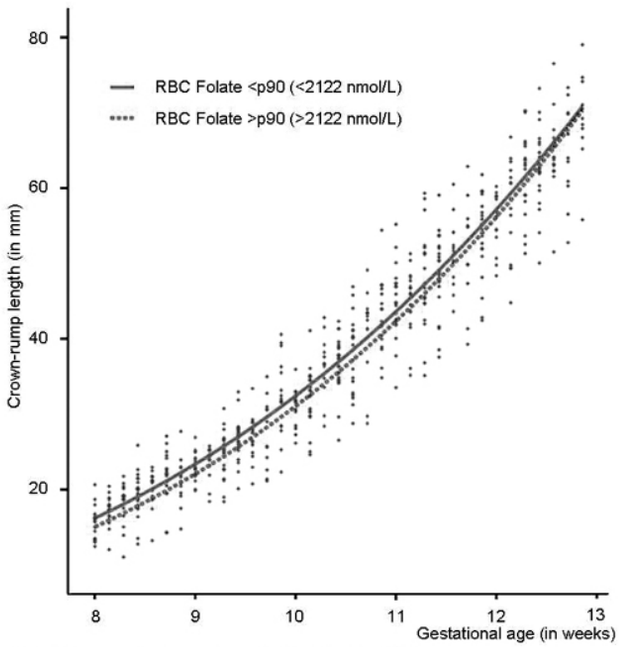


Fig 1: Regression lines for RBC folate for CRL versus gestational age

Conclusion

This study shows for the first time that high periconception maternal folate status is associated with a smaller embryo, but catch up growth nearly normalized CRL at the end of the first trimester. Whether these effects of high folate exposure are beneficial or harmful requires further investigation.

F-082

Age-Specific Contributions of VEGF to Hypoxic Reorganization of Contractile Proteins in Fetal and Adult Ovine Carotid Smooth Muscle. Olayemi O Adeoye, James M Williams, William J Pearce. *Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

OBJECTIVE: Hypoxic remodeling of arterial composition, structure, and function is well described, but the mechanisms involved remain unclear. Because hypoxia potently increases production of Vascular Endothelial Growth Factor (VEGF), which we have recently shown can modulate smooth muscle phenotype, the present study explores the hypothesis that VEGF contributes to hypoxic vascular remodeling.

METHODS: Adult and fetal sheep were acclimatized at sea level or at altitude (3280m) for the last 110 days of gestation. Freshly dissected endothelium-denuded carotid arteries from term fetuses and non-pregnant adults were: 1) mounted in vitro for contractility studies; 2) frozen for immunoblots of Smooth Muscle alpha-Actin (SMaA), Myosin Light Chain Kinase (MLCK), and 20 kDa Regulatory Myosin Light Chain (RLC); or 3) fixed in 4% paraformaldehyde and sectioned for confocal colocalization analysis of interactions among MLCK, RLC and SMaA. In parallel, endothelium-denuded carotids from normoxic animals were organ-cultured 24h in a physiological concentration of VEGF (3 ng/ml), and then were processed as described for the fresh arteries.

RESULTS: Chronic hypoxia significantly increased wall thickness (F: 15%, A: 23%), stiffness (F: 13%, A: 24%), and expression of SMaA (F: 7%, A: 47%) and RLC (F: 89%, A: 82%). Hypoxia also increased colocalization for fetal MLCK-SMaA (42%), RLC-SMaA (F: 132%, A: 805%) and MLCK-RLC (F: 492%, A: 335%) but significantly depressed myogenic tone (F: 74%, A: 40%) and MLCK expression (F: 99%, A: 97%). Organ culture with VEGF increased expression of SMaA up to 15% (AH), RLC up to 15% (FN & FH) and MLCK up to 133% (FN). VEGF also increased colocalization for MLCK-SMaA (F: 138%, A: 86%), RLC-SMaA (F: 230%, A: 410%), and MLCK-RLC (F: 100%).

CONCLUSION: As for hypoxia, VEGF effects were similarly age-dependent. Overall, there was no direct association between protein abundance and colocalization, indicating that both hypoxia and VEGF specifically reorganize contractile protein distribution within arterial smooth muscle. For both hypoxia and VEGF, changes in contractility were most closely associated with changes in MLCK-RLC colocalization. Together, these results support the hypothesis that VEGF contributes to hypoxic vascular remodeling and further suggest that contractile protein reorganization is a major component of hypoxic vascular remodeling.

F-083

Bivariate Phase-Rectified Signal Averaging (BPRSA): Description of Novel Technique. A Bauer,[1] D Casati,[2] T Stampalija,[2] C Zanardi,[2] E Rosti,[2] C Mastroianni,[2] V Signorelli,[2] S Zullino,[2] E Ferrazzi.[2] *[1]Cardiology, University of Tuebingen, Germany; [2]Obstetrics, Childrens' Hospital Buzzi, University of Milan, Italy.*

Non-stationarities are a major problem in the analysis of long recordings of complex biologic signals. Many internal/external perturbations cause interruptions of the periodic behavior and lead to phase de-synchronization of the oscillations. Phase-Rectified Signal Averaging (PRSA) showed the ability to extract *quasi*-periodic components out of non stationary, noisy time series. Bivariate PRSA allows the analysis of the interrelations between two synchronously recorded biologic signals that -supposedly- influence each other.

Methods: BPRSA technique analyzes the variations in one signal (target) when another signal (trigger) is in a certain phase/state. It is based on 4 steps: 1.Selection of anchor points in target signal contemporary to increase/decrease in trigger signal; 2.Definition of segments around anchors; 3.Phase-rectification and 4.Averaging of all segments. We applied BPRSA to evaluate the coupling between uterine activity (UA), as trigger signal, and fetal R-R intervals (fRR), as target signal, during labor. The signals were simultaneously recorded by transabdominal ECG (ta-ECG) (MonicaAN24).

Results: It was possible to apply BPRSA analysis to 104 raw data recorded by ta-ECG overcoming the problems of non-stationarity, non-linear relationship, noise and artifacts. The presence/absence of coupling between UA and FHR was assessed 'condensing' long time series in one, clear, mathematically computable (and so reproducible) graph showing (*quasi*)periodicities of the target signal coupled to the trigger signal.

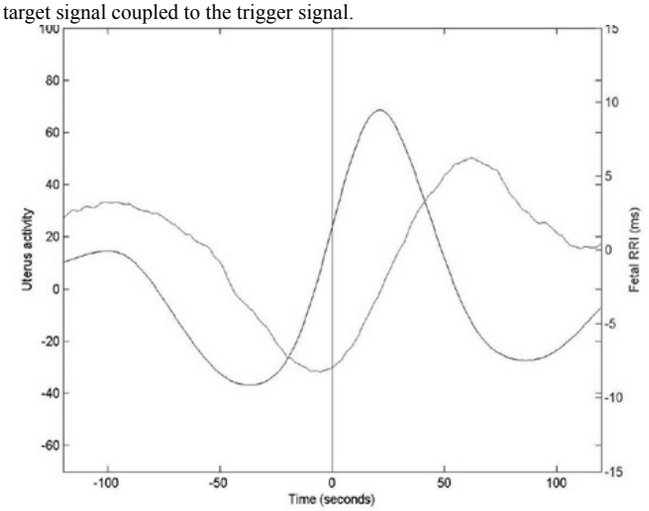


Figure 1. BPRSA: coupling between UA-trigger (blue) and fRR-target (green). The time axis allows to see how signals oscillate before and after the trigger event.

Conclusions: This is the first description of BPRSA application in obstetrics. BPRSA could represent a powerful tool for the analysis of interelationships between two or more synchronously recorded signals generated by complex biologic systems. Further studies are needed to explore the clinical usefulness of these findings.

F-084

Coupling between Fetal Heart Rate and Uterine Activity Analyzed by Bivariate Phase-Rectified Signal Averaging (BPRSA). D Casati,[1] T Stampalija,[1] A Bauer,[2] C Zanardi,[1] E Rosti,[1] C Mastroianni,[1] V Signorelli,[1] S Zullino,[1] E Ferrazzi.[1] *[1]Obstetrics and Gynecology, Childrens' Hospital Buzzi, University of Milan, Italy; [2]Cardiology, University of Tuebingen, Germany.*

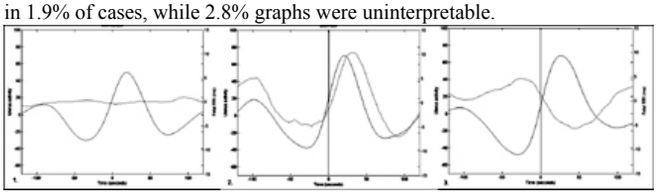
Fetal heart rate (FHR) variability is the main proxy of fetal wellbeing during labor, being an indirect mirror of oxygenation and integrity of the autonomic nervous system and its ability to respond to internal and external stimuli.

Aim: To evaluate the correlation (coupling) between FHR and uterine activity (UA) by Bivariate PRSA analysis, as a potential novel tool in clinical obstetrics.

Methods: Electrohisterogram, maternal and fetal ECG were simultaneously recorded by transabdominal ECG during the first stage of labor in women with uneventful pregnancy. BPRSA analysis (SGI 2012: Bivariate Phase-Rectified Signal Averaging (BPRSA): Description of Novel Technique) was applied to fetal RR-intervals (fRR) (target signal) in ascending phase of electrical UA

Friday

(contraction) (trigger signal). Graphical representation was obtained for each recording. Different patterns of coupling were evaluated by qualitative analysis. Results: 104 graphs were analyzed. Each graph compresses long time series of two related biological signals in one clear image. In 95.2% of cases there was presence of coupling with 2 different patterns: accelerative (50,5%) or decelerative (49,5%) response of FHR to UA. Coupling could not be identified in 1.9% of cases, while 2.8% graphs were uninterpretable.



BPRSA graphs: Absence of coupling (1) between UA-trigger (blue oscillatory line), and fRR-target (green flat line); Presence of coupling (green line oscillation): decelerative (2) and accelerative (3) patterns.

Conclusions: As previously described, BPRSA analysis brings number of potential advantages over "standard" techniques. We were able to demonstrate the presence of coupling between FHR periodicity and uterine contractions identifing a cause-effect relationship between two complex biological signals. This new technique opens the door to future insights in fetal-maternal wellbeing monitoring in labor, with the need for further studies to explore the clinical usefulness.

F-085

Ketamine Decreases Plasma Adrenocorticotropic Hormone (ACTH) Levels in Late Gestation Fetuses Exposed to Global Acute Hypoxic Hypoxia (HH). Eileen I Chang, Charles E Wood. *Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*

Introduction: Ketamine, a noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist, is a common pediatric anesthesia and analgesia for pre-term and neonates due to its pharmacological and physiological effects. Previously, we have shown that ketamine inhibits plasma ACTH levels in late gestation fetal sheep subjected to brachiocephalic occlusion (BCO), a potent stimulant of hypothalamic-pituitary-adrenal (HPA) axis activated by chemoreceptors responding to hypoperfusion. Fetal HPA axis is activated under stressful situations like HH, and treatment with ketamine could decrease chemoreceptor activation, consequently, reducing the HPA axis activity. We propose that treatment with ketamine will reduce fetal ACTH levels when exposed to acute HH.

Methods: Fetal sheep were chronically catheterized at gestational day 125 (n=3-5/group), and tracheostomy was performed on the ewe. Ketamine (3 mg/kg) was administered intravenously to the fetus 10 min prior to maternal hypoxic scenario; fetal HH was induced by administering nitrogen gas directly to the ewe for 30 min. Maternal and fetal blood gases, and fetal heart rate and blood pressure were continuously monitored and recorded throughout the experiments. Plasma samples were collected for hormonal analysis.

Results: HH stimulation significantly increased both fetal ACTH and cortisol levels in both control and ketamine groups ($P<0.0001$, main effect of time in two-way ANOVA). Ketamine treated fetuses have significantly reduced ACTH levels versus the control ($P<0.005$, main effect of groups in two-way ANOVA). The interaction effect is also statistically significant for fetal ACTH ($P<0.001$, two-way ANOVA). Fetal cortisol levels were higher in ketamine treated group versus the control, but no significance between the two groups.

Conclusion: Ketamine reduced fetal ACTH responses to HH, possibly due to antagonism of the NMDA receptors in the fetal brain. Interestingly, in contrast to the responses to BCO, ACTH responses to HH were only partially inhibited, suggesting that multiple neurotransmitter pathways mediate the ACTH response to HH. The lack of inhibition of cortisol, consistent with ketamine-BCO experiments, suggests that there are dynamic changes in adrenal sensitivity in response to HH that are not inhibited by ketamine. Alternatively, the increases in fetal plasma cortisol during HH could originate in the maternal HPA axis.

F-086

Myocardial Thyroid Hormone Deiodinases Are Regulated by Fetal T_3. Natasha N Chattergoon,[1] Samantha Louey,[1,2] George D Giraud,[1,2,3] Kent L Thornburg.[1,2] *[1]Heart Research Center, Oregon Health and Science University, Portland, OR, USA; [2]Medicine (Cardiovascular Medicine), Oregon Health and Science University, Portland, OR, USA; [3]Cardiology, Portland Veterans Affairs Medical Center, Portland, OR, USA.*

OBJECTIVES: Conversion of prohormone T_4 (thyroxine) to active tri-iodo-L-thyronine (T_3) involves the removal of one iodine under the catalytic action of the deiodinase enzyme, D2. D1 also stimulates conversion to active T_3 while D3 deactivates T_3. Current dogma holds that the near term surge in cortisol regulates deiodinase expression in the fetal liver to facilitate the T_3 surge just prior to birth. However, regulation of the deiodinases in the heart by the two powerful fetal cardiac growth regulating hormones, T_3 and insulin-like growth factor-1 (IGF-1) has not been studied. T_3 promotes maturation while IGF-1 promotes proliferation in the fetal heart. We hypothesized that T_3 and IGF-1 would not influence the expression of the deiodinases in the heart because the control of T_3 levels would logically be solely under the control of circulating fetal cortisol.

METHODS: Fetal sheep were instrumented at 120 days of gestation (dGA, term ~145dGA). Fetuses were randomly assigned to 4 groups (n=6 each group) and studied from 125-130dGA: 1) Long R^3 IGF-1 (IGF-1, 715µg/d), 2) T_3 (54µg/d), 3) IGF-1 combined with T_3 (T_3+IGF-1), and 4) control (CON). At 130dGA, a section of the fetal heart was snap frozen for tissue analysis. Gene expression of the three deiodinases was analyzed by qPCR.

RESULTS: T_3 stimulated expression of all three deiodinases compared to controls ($p<0.05$). IGF-1 alone did not effect expression of any of the deiodinases. While the combination of T_3 and IGF-1 exposure did not alter D1 expression, D2 and D3 levels were decreased compared to T_3 alone ($p<0.05$). CONCLUSIONS: Contrary to our hypothesis, T_3 increased expression of all 3 deiodinases. IGF-1 did not alter deiodinase expression compared to CON; T_3 + IGF-1 suppressed D3 while not affecting D1 expression compared to T_3 alone. The presence of IGF-1 blunted D3 expression increasing T_3's availability; T_3's action is dominant over IGF-1 and would promote maturation of fetal cardiomyocytes. In the developing heart T_3 is affected by a feed forward system that upregulates deiodinase enzymes and likely leads to premature maturation of cardiomyocytes. The extent to which D1 and D3 deiodinate and reduce T_3 concentrations needs further investigation. Supported by NICHD and NHLBI.

F-087

Leptin Antagonist Treatment Enhances Expression of CYP17 and CYP11A1 in the Adrenal Cortex of Long Term Hypoxic (LTH) but Not Normoxic Late Gestation Fetal Sheep. C Ducsay,[1] K Kaushal,[1] K Hyatt,[2] K Hanson,[2] K Furuta,[1] D Myers.[2] *[1]Ctr. Perinatal Biol., Loma Linda Univ.; [2]OB/GYN, Oklahoma Univ. HSC.*

BACKGROUND: The LTH ovine fetus has a normal late gestation adrenocortical maturation despite elevated fetal plasma ACTH concentrations, indicative of critical adaptive responses. We reported elevated adipose expression, plasma leptin concentrations and adrenocortical expression of the leptin receptor in the LTH fetus. However, leptin's role in mediating the adaptive responses in the adrenal cortex remains unresolved. This study was designed to determine if leptin receptor antagonist (LRA) administration to late gestation LTH fetal sheep altered expression of key genes governing cortisol synthesis.

MATERIALS AND METHODS: : Pregnant ewes were maintained at high altitude (3,820 m) from day 40 to ~130 days gestation (dG) when a maternal tracheal catheter was implanted. Reduced PO_2 was maintained by nitrogen infusion. On day 132, LTH (n=12) and age-matched, normoxic control (n=8) fetuses underwent vascular catheter implantation. At 138 dG, fetuses were infused with either saline or LRA (1.5mg/kg/day) for five days continuously, at which time, fetal adrenal cortex was collected. StAR, CYP17 and CYP11A1 mRNA were measured by qRT-PCR. Data are expressed in fg mRNA/ 50 ng RNA, mean±SEM.

RESULTS: StAR (LTH: 8.7±1.08 vs. CONT: 14.2±1.65), CYP17 (LTH: 6.39±0.37 vs. CONT: 11.98±1.8) and CYP11A1 (LTH: 3.09± 0.16 vs. CONT: 5.32±0.38) were significantly ($p<0.05$) lower in LTH compared to control fetuses infused with saline. In contrast, LRA infusion elevated CYP17 and CYP11A1 mRNA in the LTH but had no effect on normoxic control fetuses: CYP17 (LTH: 9.13±1.11 vs. CONT: 9.13±1.44) CYP11A1, (LTH: 5.10±0.43 vs. CONT: 5.10±0.43). LRA increased StAR mRNA to a level similar to normoxic control fetuses but not significantly elevated from LTH infused with saline (LTH: 11.18±1.21 vs. CONT: 11.48.13±1.58).

CONCLUSIONS: LRA had no effect on adrenocortical expression of key enzymes and StAR in normoxic control fetuses despite previous reports that pharmacologic leptin administration suppresses the HPA axis during late gestation. In contrast, in the LTH fetus, LRA returned enzyme expression to that of age matched normoxic fetal sheep. Considering the elevated ACTH levels in the LTH fetus, leptin plays a role in the adaptive response, but clearly, other factors are also involved in this critical adaptive response. (HD31226,P20-MD001632)

F-088

Continuous Fetal Heart Rate Variability (fHRV) Analysis Allows for Early Detection of Hypoxic-Acidemia Near-Term. D Durosier,[1] M Cao,[1] G Green,[2] I Batkin,[2] A Seely,[2] B Richardson,[3] M Frasch.[1] *[1]OBGYN, U de Montréal, QC, Canada; [2]OHRI, U of Ottawa, ON, Canada; [3]OBGYN, UWO, London, ON, Canada.*

Objective: FHRV measure RMSSD reflects vagal activity and increases during severe acidemia (pH ~7.09) in near-term fetal sheep(doi:10.1177/1933719108327597). We hypothesized that continuous FHRV monitoring would allow for earlier detection of worsening hypoxic-acidemia as might occur in human labour. We also tested the performance of RMSSD as currently available clinically (4 Hz sampling rate).

Methods: Near-term ovine fetuses (N=7) were chronically prepared with vascular catheters and umbilical cord occluder. For 1 min every 2.5 min, animals underwent mild partial UCO x 1 h, moderate partial UCO x 1h, then complete UCO x 1-2 h, until arterial pH reached < 7.00. Arterial blood samples were drawn at baseline, every 20 min during the UCO series and at 1 h of recovery. RMSSD was calculated continuously in 5 min windows using an automated, standardized system (CIMVA.org). Averaged RMSSD values over 20 min intervals of baseline, UCO series and 1 h recovery were correlated to time-matched pH, lactate and base excess (BE) values. Results are presented as mean±SEM for $p<0.05$.

Results: Repetitive UCO resulted in pH decreasing from 7.36±0.01 to 6.99±0.01. In all 7 animals, baseline RMSSD of 12.8±1.8 ms increased to 41.4±6.3 ms at 70±15 min (pH 7.16±0.04) prior to reaching pH nadir. This increase persisted until 1 h recovery. RMSSD correlated to pH (R=-0.60), lactate (R=0.74) and BE (R=-0.52) (all $p<0.0001$). When measured at 4 Hz fHRV sampling rate, baseline RMSSD was 52.2±26.3 ms, increasing in 6 out of 7 animals at 60 and 100 min to 73.0±11.3 and 102.2±9.1 ms, respectively. Detection of severe hypoxic-acidemia was now only possible 30±13 min (pH 7.12±0.04) prior to pH nadir and 2 fetuses would have been missed. RMSSD still correlated to pH (R=-0.39), lactate (R=0.57) and BE (R=-0.29) (all $p<0.01$).

Conclusion: Confirming our hypothesis RMSSD allowed for the early detection of worsening hypoxic-acidemia in each fetus and correlated well with pH, lactate and BE. While the simulated low clinical FHRV sampling rate still showed an RMSSD increase, the correlation to measures of metabolic acidosis was weaker and the detection of hypoxic-acidemia was delayed ~2fold. This is due to an overestimating of fHRV due to its undersampling. Thus, for earlier detection of fetal acidemia during labour, more sensitive means of acquiring FHR are recommended than currently deployed.

F-089

Chronic Intrauterine Hypoxia Activates MEK/ERK1/2 Pathway Via iNOS-Derived NO in Isolated Fetal Guinea Pig Cardiomyocytes. LaShauna C Evans,[1] Hongshan Liu,[2] Gerard A Pinkas,[2] Loren P Thompson.[2] *[1]Depts of Physiology, Univ. of Maryland; [2]Obstet, Gynecol & Repro Sci, Univ. of Maryland SOM, Baltimore, MD.*

We have previously shown that prenatal hypoxia induces oxidative stress in fetal guinea pig hearts. This is associated with an increase in inducible Nitric Oxide Synthase (iNOS)-derived NO and peroxynitrite levels, presumably from a NO/O_2^- interaction. Additionally, peroxynitrite activates matrix metalloproteinases (MMPs) in hypoxic fetal guinea pig heart ventricles, which then has downstream actions on collagen synthesis and extracellular matrix remodeling. Previous studies have reported that MMPs can be acted on by upstream signaling proteins of the MAPK pathway in adult heart pathologies. To determine the role of the MAPK pathway in hypoxic fetal hearts, we tested the hypothesis that intrauterine hypoxia activates MEK/ERK1/2 in fetal heart cells via iNOS-derived NO generation.

Methods: Pregnant guinea pigs were exposed to room air (normoxic, NMX) (n=4) or 10.5% O_2 (hypoxic, HPX) (n=4) for 14d prior to term (term=65d). In separate animals, L-N6-(1-Iminoethyl)-Lysine (L-NIL), a selective iNOS inhibitor, was administered (1-2mg/kg/d) to pregnant NMX (n=4) and HPX (n=4) mothers via their drinking water. At 63d gestation, near-term fetuses were removed via hysterotomy from anesthetized sows. Fetal hearts were excised and cardiomyocytes were isolated and stored at -80°C. MAP kinase proteins of p-MEK, p-ERK, and total ERK were measured by Western and normalized to b-actin protein. **Results:** Chronic hypoxia increased ($P<0.05$) protein expression of p-MEK by 53% (0.84±0.11 vs 1.28±0.11, NMX vs HPX, respectively) and p-ERK by 77% (0.54±0.08 vs 0.96±0.09) compared to NMX controls. L-NIL reversed ($P<0.05$) the hypoxia-induced increase in both p-MEK (1.18±0.11 vs 0.69±0.11, HPX vs HPX+L-NIL, respectively) and p-ERK (1.61 0.12 vs 1.06±0.06) protein levels by 41% and 34%, respectively, compared to HPX alone. Neither HPX nor L-NIL had any effect on protein levels of total ERK.

Conclusions: These results suggest that iNOS-derived NO mediates hypoxia-induced activation of the MEK/ERK1/2 signaling pathway in isolated cardiomyocytes. This identifies an important downstream signaling pathway (i.e. MAPK) activated by NO that could contribute to cardiac growth and remodeling in the hypoxic fetal guinea pig. (HL49999/LT and HL90044/LE).

F-090

Fetal Sheep Electrocorticogram (ECOG) and Electroencephalogram (EEG) Changes Accompanying Variable Fetal Heart Rate (FHR) Decelerations Warn Early of Acidemia. M Frasch,[1] D Durosier,[1] C Duchatellier,[1] B Richardson.[2] *[1]OBGYN, U de Montréal, QC, Canada; [2]OBGYN, UWO, London, ON, Canada.*

Objective: Fetal sheep ECOG is predictably altered with worsening hypoxic-acidemia and FHR decelerations and might prove useful for monitoring fetal health during labour (Frasch et al PLoS ONE 2011). Fetal sheep EEG recorded from a modified scalp electrode under normoxic conditions near term shows similar frequency properties as fetal ECOG. Here we compare the fetal EEG and ECOG responses to repetitive FHR decelerations with worsening acidemia. We hypothesize that these will show similar properties and predictive value for severe acidemia.

Methods: Near-term ovine fetuses (N=7) were chronically prepared with vascular catheters and umbilical cord occluders. For 1 min every 2.5 min, animals underwent 1 h mild partial UCO, 1 h moderate partial UCO, and 1-2 hours of severe complete UCO until arterial pH reached < 7.00. Arterial blood samples were drawn at baseline and every 20 min during the UCO period. EEG/ECOG amplitude and 95% spectral edge frequency (SEF) were analyzed in 4 s sliding windows and compared using cross-correlation function (CCF) analyses. The closer CCF maxima (CCFM) are to 1, the higher is the correlation between both signals. Timing of the appearance of changes in ECOG/EEG in relation to arterial pH was recorded.

Results: Repetitive fetal UCO resulted in marked acidosis (pH 7.36±0.01 to 6.99±0.01). For ECOG, at a pH of 7.16±0.04, 65±16 min prior to the pH dropping <7.00, SEF increased to 8.4±0.8 Hz from 5.4±0.4 Hz ($p<0.05$) and amplitude decreased ~4fold ($p<0.01$) during each FHR deceleration and was correlated to decreases in arterial blood pressure (ABP) ($R=1.00$, $p<0.001$). For EEG, at a pH of 7.14±0.03, 58±14 min prior to the pH dropping <7.00, SEF remained unchanged averaging 5.7±0.6 Hz, while amplitude also decreased ~4fold ($p<0.01$) during each FHR deceleration and was correlated to decreases in ABP ($R=0.96$, $p<0.001$). While baseline EEG amplitude was ~2x lower than ECOG amplitude, it was similar during the UCO series and EEG/ECOG 95% SEF values were highly correlated with an overall CCFM value of 0.86±0.02.

Conclusion: These findings provide proof of principle for fetal EEG monitoring during high-risk human labour. EEG recorded from the scalp of near-term fetal sheep shows changes comparable to ECOG during worsening hypoxic-acidemia. Fetal EEG monitoring may be a useful adjunct to FHR monitoring.

F-091

Regional Difference of Protecting Effect of Acetylcholine Receptor Agonist on Brain Damage Induced by Hypoxia-Ischemia in Newborn Rats. Seishi Furukawa, Li Yang, Hiroshi Sameshima, Tsuyomu Ikenoue. *Obstetrics & Gynecology, University of Miyazaki, Faculty of Medicine, Miyazaki, Japan.*

Objective: The newborn rat model has been developed to elucidate the mechanism and the management of perinatal brain damage. Our purpose is to elucidate the effect of acetylcholine receptor agonist on white matter brain damage by hypoxia-ischemia (HI) in the newborn rat model.

Study design: 7-day-old Wistar rats were divided into 2 groups at random: carbachol (acetylcholine receptor agonist) single pre-injection and HI (Carb/HI) and saline pre-injection and HI (Saline/HI). Rats were subjected to left carotid artery ligation followed by 2 hours of hypoxia (8% oxygen). We injected carbachol or saline before hypoxic loading. After 24, 48, and 72 hours, we checked for brain damage. Comparisons were made with χ^2 test. Probability values < 0.05 were considered significant.

Results: In the hippocampal regions, more than 66% of the Carb/HI group showed mild neural damage on 24, 48 and 72 hours after HI ($p=0.70$). In contrast, no more than 30% of the Saline/HI group had mild neural damage on 24 and 48 hours ($p=0.02$). In the white matter regions, tissue damage gradually increased with time course in Carb/HI group ($p<0.01$). 90% of Carb/HI showed mild tissue damage on 24 hours. 45% on 48 hours and 9% on 72 hours in Carb/HI showed mild damage. In contrast, tissue damage of Saline/HI group abruptly increased with time course ($p=0.01$). 50% of Saline/HI showed mild damage on 24 hours. No more than 20% showed mild damage on 48 and 72 hours after HI.

Friday

Conclusion: In opposite to hippocampus, carbachol did not prevent progression of white matter brain damage induced by hypoxia-ischemia in newborn rats. The brain protective effect of acetylcholine receptor agonist against HI is different at each region.

F-092

Fetal and Maternal Intima Media Thickness (IMT): A Feasibility Study. Sander Galjaard,[1] Agnieszka Zawiesjska,[2] Ewa Wender-Ozegowska,[2] Roland Devlieger.[1] *[1]Department of Obstetrics and Gynaecology, University Hospitals KULeuven, Leuven, Belgium; [2]Department of Obstetrics and Gynaecology, University of Medical Science Poznan, Poznan, Poland.*

BACKGROUND AND AIM

We aimed to develop and investigate a methodology for a non-invasive monitoring of maternal and fetal vascular wall properties during pregnancy.

METHODS

We measured IMT in 42 pregnancies of women attending the prenatal care unit at the University Hospital of Leuven for routine ultrasound scans. The measurements were performed with a Mindray M7 high resolution ultrasound-machine, equiped with a 3.0-7.0 MHz linear array transducer and automated IMT measurement software. IMT was measured in a coronal or sagittal plane at predefined locations of fetal and maternal arterial vessels. The fetal vessels investigated included the abdominal aorta, renal artery, common carotid artery and the umbilical artery. The maternal vessels included the uterine artery, external iliac artery, abdominal aorta and the common carotid artery.

RESULTS

In maternal vessels IMT measurement of carotid artery was feasible in all cases, but the external iliac artery was not always feasible. In the abdominal aorta and the uterine artery it depended on gestational age. In fetal vessels IMT measurements of the abdominal aorta and umbilical artery were increasingly feasible with increasing gestational age. IMT in Fetal carotid and renal artery was not always measurable, not even at more advanced gestations.

Table 1. IMT feasibility (%) of assessed vessels and range (µm)

Vessels assessed	1st Trimester N = 5	Range (µm)	2nd Trimester N = 14	Range (µm)	3rd Trimester N = 23	Range (µm)
Maternal						
MABDAo	40%	523-616	50%	382-612	39%	495-732
MCCARArt	100%	400-516	100%	440-712	100%	378-740
MIlExtArt	-		-		-	
MUtArt	40%	163-415	57%	195-601	61%	176-535
Fetal						
FABDAo	-		71%	181-431	96%	201-561
FCCARArt	-		-		17%	185-367
FRenArt	-		-		-	
UA	-		50%	225-572	78%	212-417

MABDAo (Maternal Abdominal Aorta), MCCARArt (Maternal Common CARotid Artery), MIlExtArt (Maternal Iliacal External Artery), MUtArt (Maternal Uterine Artery), FABDAo (Fetal ABDominal Aorta), FCCARArt (Fetal Common CARotid Artery), FRenArt (Fetal Renal Artery) and UA (Umbilical Artery)

DISCUSSION

Our study shows that IMT is feasible in some maternal and fetal vessels of interest. Further studies of IMT in normal and abnormal pregnancies are necessary to obtain more insight in the vascular development during normal and pathologic pregnancy.

F-093

Chronic Hypoxia during Pregnancy Suppresses Large-Conductance Ca^{2+}-Activated K^+ Channel in Ovine Uterine Arteries. Xiang-Qun Hu, Lubo Zhang. *Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Introduction: Chronic hypoxia during pregnancy is a major stress to maternal cardiovascular homeostasis and has profound adverse effects on fetal development. Previous studies demonstrated that chronic hypoxia during pregnancy significantly increased pressure-dependent myogenic tone of resistance-sized ovine uterine arteries. However, the underlying mechanisms are not fully understood. Given that large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel plays a key role in regulation of myogenic tone, we hypothesized that chronic hypoxia during pregnancy suppresses activity of BK_{Ca} channels in ovine uterine arteries.

Methods: Whole-cell patch-clamp recording was performed on myocytes enzymatically isolated from resistance-sized uterine arteries of nonpregnant (NPUA) and near-term pregnant (PUA) sheep maintained at sea level (altitude: ~300 m; PaO_2: 102±2 mm Hg) or at high altitude (altitude: 3801 m; PaO_2: 60±2 mm Hg) for ~110 days.

Results: Pregnancy caused a significant increase in whole-cell K^+ current (NPUA, 37.5±3.3 pA/pF; PUA, 66.3±5.2 pA/pF, P<0.05). The inhibition of the K^+ current by tetraethylammonium (TEA), a selective BK_{Ca} channel blocker, was greater in PUA than in NPUA (53.2±1.8% versus 38.5±2.5%, P<0.05). Similar findings were obtained with a specific BK_{ca} channel blocker iberiotoxin. Accordingly, BK_{Ca} current was greater in PUA than in NPUA (35.1±2.9 versus 14.1±1.3 pA/pF, P<0.05). Exposure to chronic hypoxia produced a reduction of whole-cell K^+ current (47.0±3.3 pA/pF), less inhibition by TEA (40.5±1.2%), and a decrease in BK_{Ca} current (19.1±1.5 pA/pF) in PUA, respectively (P<0.05), but not in NPUA (40.2±2.8 pA/pF, 35.3±1.7%, and 14.1±1.1 pA/pF, respectively, P>0.05). The enhanced BK_{Ca} channel activity seen in PUA from normoxic animals was mimicked by treatment of NPUA with 17β-estradiol and progesterone for 48 hours in an ex vivo tissue culture system. This treatment resulted in a 2.5-fold increase in BK_{Ca} current density (24.5±3.7 pA/pF versus 10.4±2.4 pA/pF, P<0.05). However, the hormonal treatment failed to upregulate BK_{Ca} channel activity in NPUA from the chronic hypoxic animals (18.4±1.8 pA/pF versus 14.0±2.2 pA/pF).

Conclusion: Our results suggest that chronic hypoxia suppresses BK_{Ca} channel activity in pregnant uterine arteries, which likely represents a mechanism of increased myogenic tone of uterine arteries during pregnancy in hypoxic animals. (Supported in part by a NIH grant HD31226)

F-094

The Influence of Hypoxia on the Expression of µ-Opioid Receptor mRNA and Enkephalin in Newborn Rat Brain. Janusz J Kraczkowski, Katarzyna M Karwasik-Kajszczarek, Jacek M Robak, Anna Kwasniewska. *Department of Obstetrics and Pathology of Obstetrics, Medical University of Lublin, Lublin, Poland.*

Background: Fetal hypoxia is considered to be a major factor causing changes in the nervous cells of the fetal brain. It has been already proved that prenatal hypoxia induces the release of endogenous opioids that modulate the growth and development of CNS and downregulates µ-opioid receptors in neonatal brain. Endogenous opioid peptides and µ- opioid receptors are present in brain structures responsible for reproductive processes and behaviors. Opioid peptides may have widespread effects as growth regulators.

Methods: We analyzed the expression of mRNA of µ- opioid receptor and the expression of enkephalin in different brain area in newborn rat brains. We exposed 12 time pregnant Sprague-Dawley dams to hypoxia for 24 h per day from days E-15 to E-20. 12 dams growing in normal conditions were used as a control. On day 21 of gestation fetal brains were extracted, cut in cryostat and mounted on gelatin and poly-L-lysine- coated slides. We used in situ hybridization to examine µ-receptor mRNA expression in neuroepithelial zones of fetal rat brain: Caudate Putamen- patch- CPu (p), Caudate Putamen-matrix- CPu (m), Ventricule Zone- VZ, Nucleus Accumbens- NA and Olfactory Tubercle- OT. Quantitative autoradiography were used to measure enkephalin expression in tissue slices.

Results: Comparing control and hypoxic group, we have found no significant differences in µ- opioid receptor mRNA expression. We have seen a significant upregulation of enkephalin expression in caudal, but not rostral caudate (p < 0,001).

Conclusions: Prenatal hypoxia may alter fetal brain development by the increase opioid peptide concentrations. We observed enkephalin release in newborn rats under hypoxic conditions. Different opioid mechanisms may regulate cell division in rostral and caudal brain regions.

µ-opioid receptors mRNA expression in brain under hypoxia conditions

Brain region	Control (dpm equivalents)	Hypoxia (dpm equivalents)
CPu (p)	1619,04 ± 59,52	1297,61 ± 173,80 (NS)
CPu (m)	1214,28 ± 142,85	1178,57 ± 166,66 (NS)
NA	2071,42 ± 119,04	2238,09 ± 142,85 (NS)
OT	1857,14 ± 166,60	1833,33 ± 178,57 (NS)
VZ	2164,28 ± 142,85	2090,47 ± 116,66 (NS)

NS- no significance

Enkephalin expression in brain under hypoxia conditions

Brain region	Control (dpm equivalents)	Hypoxia (dpm equivalents)
CPu (r)	1704,2 ± 35,2	1676,0 ± 70,5 (NS)
CPu (c)	3098,5 ± 70,4	4190,1 ± 281,6 *

* p < 0,001

F-095

Maternal Supraphysiological Hypercholesterolemia Leads to Reduced Endothelium-Dependent Vasodilation of Human Umbilical Vein and Reduced Nitric Oxide Production in HUVEC. Andrea A Leiva, Enrique Guzman-Gutierrez, Fernando Abarzua, Paola Casanello, Luis A Sobrevia. *Obstetrics and Gynecology, Pontificia Universidad Católica de Chile, Santiago, Chile.*

Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal growth and development but maternal supraphysiological hypercholesterolemia (MSPH) leads to aortic atherosclerosis in the fetus and children. Since nitric oxide (NO) synthesis is reduced in atherosclerosis, we hypothesize that MSPH will alters L-arginine transport (NO synthases (NOS) substrate) and NO synthesis in human umbilical vein endothelial cells (HUVEC) leading to altered vascular reactivity. **Aim.** To estimate MSPH incidence in Chilean population and the effect of this condition in placental endothelial function. **Methods**. MSPH incidence was estimated considering a cut-point >280 mg/dl for maternal blood cholesterol in the third trimester of pregnancy in a population of pregnant women (n=56) of Hospital Clínico UC (Santiago de Chile). Umbilical vein rings from women with MPH and MSPH were mounted in a myograph and endothelium- dependent (calcitonin gene-related peptide, CGRP, 10^{-10} -10^{-7} M) or independent (sodium nitroprusside, SNP, 10^{-5} M) vasodilatation was determined. L-Arginine transport (30-500 μM, 3 μCi/ml, 37ºC, 1 minute) and L-[^{3}H]citrulline formation from L-[^{3}H]arginine (9 μCi/ml, 60 minutes, 37ºC) in absence or presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) was measured in HUVEC. **Results**. A mean of 263 ± 5.2 mg/dl of cholesterol was determined for the third trimester of pregnancy with 95th percentile on 342 mg/dl. MSPH incidence at term was 40.4%. MSPH was associated with reduced CGRP-relaxation (IC_{50} 3.2 ± 0.3 nM) compared with MPH (IC_{50} 0.15 ± 0.002 nM); however, SNP-vasodilatation was unaltered in MSPH. L-Arginine transport maximal velocity was higher in MSPH compared with MPH (V_{max} 12 ± 3 and 5 ± 1 pmol/μg protein/minute respectively) without significant changes in apparent K_m (188 ± 72 and 128 ± 54 μM, respectively). NOS activity was lower (29%) in MSPH compared with MPH without changes in eNOS expression. **Conclusion**. We propose that MSPH associates with altered L-arginine bioavailability for NOS in HUVEC leading to reduced umbilical vein reactivity, a likely key phenomenon in MSPH-associated adult cardiovascular disease.

F-096

Is Umbilical Artery Doppler a Good Indicator of Fetal Well Being in Gastroschisis? Bethany Lee Hart, Stephanie E Mann. *Gynecology and Obstetrics, University at Buffalo.*

Objective: Intrauterine growth restriction is a common complication of fetal gastroschisis; however, there is limited evidence on the interpretation of umbilical artery doppler indices in these fetuses. The purpose of this study is to evaluate umbilical artery Doppler indices in gastroschisis fetuses that are growth restricted and those that are growing normally to determine if there is any difference between these two groups with respect to standard measures of neonatal well being.

Methods: This retrospective study included all fetuses diagnosed and delivered with gastroschisis at our regional perinatal center between 2000-10. Incomplete charts or fetuses with other conditions or abnormal karyotypes were excluded. Fetal growth restriction was defined as an EFW < 10th %tile. Variables that were measured included umbilical artery S/D ratio, PI, and RI, birthweight, five minute apgar score, and umbilical artery pH. A t test and Mann-Whitney tests was used to compare groups. $P < 0.05$ was considered significant.

Results: As seen in the table, for those fetuses identified prior to delivery as growth restricted, there was no difference in umbilical artery Doppler indices prior to delivery, birthweight, umbilical artery pH or apgar score at 5 min.

Conclusions: The growth restriction observed in fetal gastroschisis appears to be unrelated to placental insufficiency as evidenced by normal Doppler indices seen in these patients. Use of umbilical Doppler for growth restricted fetuses is not a predictor of fetal well-being as there was no difference in outcomes between either group.

F-097

Postnatal Fatty Acid β-Oxidative Genes Are Differentially Expressed Depending on the Cause of Fetal Growth Restriction and Sex. XY Loke,[1] KJ J Botting,[1,2] SP Seng,[1] JL Morrison.[1,2] *[1]Early Origins of Adult Health Research Group, Sansom Institute for Health Research, University of South Australia, SA, Australia; [2]Discipline of Physiology, The University of Adelaide, SA, Australia.*

Fetal growth restriction (FGR) is associated with an increased risk of cardiovascular disease in adulthood. In postnatal life, fatty acid (FA) β-oxidation accounts for ~ 80% of cardiac ATP production. Maternal protein restriction in pregnant rats altered cardiac FA content and increased peroxisome proliferator-activated receptor α (PPARα) gene expression in week old offspring. We hypothesise that FGR as a result of reduced maternal nutrition (MNR) and/or maternal oxygen (MH) will increase the expression of genes in the FA β-oxidation pathway in the heart of adult offspring.

Date-mated IMVS tri-coloured guinea pigs at 35d gestation (term, 69d) were randomly assigned to Control (C; 21% O_2 with *ad libitum* food, n=10), MH (12% O_2 with *ad libitum* food, n=7) or MNR (21% O_2 with food weight matched to daily food intake/body weight of MH, n=9). All pregnant sows birthed spontaneously in normoxia with food *ad libitum*. The heart was collected at 120d of age. The mRNA expression of fatty acyl-CoA synthase (ACS), fatty acid binding protein (FABP), carnitine palmitonyl transferase-1b (CPT1b), malonyl-CoA decarboxylase (MCD), acetyl-CoA carboxylase (ACC), AMP-activated protein kinase (AMPK), medium chain and long chain acyl-CoA dehydrogenase (ACADM and ACADL) were determined by real time RT-PCR. Data is presented as mean±SEM and analysed by two way ANOVA followed by Duncan post hoc tests.

Both MH and MNR reduced birth weight. There was no effect of treatment on the expression of ACS, FABP, CPT1b, MCD, ACC or ACADM. MH and MNR decreased expression of AMPK in male offspring (C; 3.39±0.08; MH 2.70±0.22; MNR 2.47±0.07), however, MNR, but not MH, increased expression of ACADL in female offspring (C; 2.06±0.25; MH 2.73±0.15; MNR 4.00±0.71). AMPK acts as a fuel gauge in the heart, regulating FA oxidation by phosphorylating ACC. A decrease in AMPK may lead to an increased activation of ACC, which synthesises malonyl-CoA and thus inhibits the shuttling of activated FAs into the mitochondria by CPT-1b. Male offspring exposed to MH and MNR may have decreased cardiac FA β-oxidation in adulthood resulting in impaired cardiac contraction. In contrast, female offspring exposed to MNR may have greater cardiac FA β-oxidation leading to greater oxygen consumption and vulnerability to ischemia in adulthood.

F-098

Sex Differences in Fetal Non-Human Primate (NHP) Heart Mitochondrial Transcripts at 0.9 Gestation (G). Susana P Pereira,[1,2] Paulo J Oliveira,[1] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Center for Neurosciences and Cell Biology, Univ. Coimbra, Coimbra, Portugal; [2]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*

Sex differences in cardiovascular diseases (CVD) are often seen in offspring exposed to suboptimal intra-uterine environments. The mechanisms underlying these differences remain enigmatic. Adult rat skeletal muscle and liver exhibit clear sex differences in mitochondrial energy metabolism. Data on sex differences in cardiac muscle mitochondrial metabolism are largely non-existent during fetal life.

We hypothesized that expression of mitochondrial oxidative phosphorylation transcripts in fetal cardiac left ventricle would differ by fetal sex at 0.9G.

Pregnant baboons of similar weight were fed standard chow (n = 10) until fetal morphometrics and free wall of the fetal cardiac left ventricle were collected at c-section at 0.9G. Samples were stored at –80°C. Quantitative PCR arrays were used to detect mRNA expression. Protein was measured by Western blot (WB). Data were analyzed using GraphPad Prism v5, with p-value < 0.05 considered significant.

Fetal body weight was slightly lower in females (F) compared to males (M). Heart:body weight ratio was not different. Expression of structural genes of the mitochondrial respiratory chain including subunits of Complex I (NDUFB5 and NDUFC1) and Complex IV (COX6C and COX18) was increased in F hearts. Transcripts involved in long-chain fatty acid beta-oxidation (CPT1B), the TIM/TOM complex (TIMM10, TIMM10B, TIMM23, TOMM20, TOMM22 and DNAJC19), mitochondrial carriers (SCL25A16, SLC25A17 and SLC25A3), the adenine nucleotide translocator (SLC25A4), mitochondrial trafficking (RHOT2), the regulators of mitochondrial distribution and morphology (MSTO1, MFN2) and superoxide metabolism (SOD1) were all increased in F left ventricle. Complex IV protein content was increased in F hearts.

Our results show at 0.9G that, compared to M, mitochondrial transcripts related to mitochondrial function and cell antioxidant defense are increased in F left

ventricle. Whether mitochondrial content is greater in the F heart is unknown. We hypothesize that these sex differences may correlate with the lower incidence of cardiovascular disease (CVD) in females later in life. These data may advance the prevention, diagnosis and treatment of CVDs in both sexes.
Supported by NIH PO1 HD023150 (MJN, PWN) and the Portuguese Foundation for Science and Technology SFRH/BD/64247/2009 (SPP,PJO).

F-099

Effects of Maternal Nutrition Restriction (MNR) on Fetal Cardiac Mitochondrial Transcripts at 0.9 G in Non-Human Primates (NHP). Susana P Pereira,[1,2] Paulo J Oliveira,[1] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Center for Neurosciences and Cell Biology, Univ. Coimbra, Coimbra, Portugal; [2]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*
Epidemiologic studies link low birth weight to predisposition to cardiovascular disease (CVD) later in life. Both sex and diet impact the incidence of CVD and other aging-associated complex disorders whose initiation is often delayed in females and in animals subjected to life long caloric restriction.
Our aim was to examine the effect of MNR on the mitochondrial transcript expression in cardiac left ventricle of fetal baboons at 0.9 G.
Pregnant baboons of similar body weight were fed [italics]ad lib[/italics] (C, n=12) or, starting at 0.16 G, 70% of *ad lib* fed C (MNR, n=9). Samples from the free wall of the fetal cardiac left ventricle (LV) were obtained by c-section at 0.9 G and stored at -80°C. Quantitative PCR array was used to determine mRNA expression. Data were analyzed using Graph Pad Prism v5, p-value < 0.05 considered significant.
Maternal body weight at c-section was not different in C mothers carrying male (M, 18.47±3.04 Kg; n=6) vs female (F, 18.17±2.23 Kg, n=6) fetuses. The loss of maternal body weight was greater in MNR mothers carrying M (4.15±0.84Kg, n=4) vs F (2.63±0.36 Kg, n=5; p=0.048). Fetal weight of C M (866.7±47.63 g) vs MNR M (712.5±39.06) approached significance (p=0.0505). Fetal heart:body weight ratio was unchanged. Sex-dependence in the nutrient sensitivity of mitochondrial transcript expression was evident when mitochondrial oxidative phosphorylation complexes and transport carriers were analyzed, particularly components of mitochondrial Complex I and ATP synthase.
Nutrient sensitive mitochondrial genes.

Group	Gene symbol
CM vs MNRM	NDUFS6, NDUFV1, ATP5A1, ATP5G3, SLC25A37, MNF2, MSTO1, BNIP3, HSP90AA1
CF vs MNF	NDUFB4, NDUFB6, NDUFB7, ATP5L, SLC25A10, MNF2, LHPP*, BID*, PMAIP1*

C, control; MNR, maternal nutrition restriction; M, male; F, female; * down-regulated
Mitochondrial genes up-regulated by MNR at 0.9 G are involved in mitochondrial dynamics and oxidative phosphorylation. In addition, MNR down-regulates transcripts involved in cell death in F but not in M fetuses. The present study provides evidence of an association between MNR and mitochondrial cardiac transcriptional remodeling in the fetus.
Supported by NIH PO1 HD023150 (MJN, PWN) and Portuguese Foundation for Science and Technology - SFRH/BD/64247/2009 (SPP,PJO).

F-100

RhoA/ROCK Signaling Pathway Is Not Implicated in the Regulation of eNOS Activation and arginase-2 Expression in Human Umbilical Vein Endothelial Cells Exposed to Hypoxia. Catalina P Prieto, Bernardo J Krause, Luis Sobrevia, Paola Casanello. *Division of Obstetrics & Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.*
Intrauterine growth restriction and hypoxia are associated with increased placental vascular resistance and altered nitric oxide (NO) synthesis. The latter can be explained in part by reduced endothelial NO synthase (eNOS) and increased arginase-2 (Arg-2) activity. Notably, several studies have shown the role of RhoA/Rho kinase (ROCK) signalling pathway controlling the activity of both eNOS (down-regulated) and Arg-2 (up-regulated). However it is not known whether the RhoA/ROCK pathway is implicated in the response elicited by hypoxia in human umbilical vein endothelial cells (HUVEC). Aim: We studied the role of the RhoA/ROCK pathway on eNOS and Arg-2 expression in HUVEC exposed to hypoxia. Methods. HUVEC primary cultures were exposed to normoxia (5% O2) or hypoxia (2% O2) for 0-24 hours. Cells were treated with fasudil (ROCK inhibitor, 10 μM) or transfected with a RhoA siRNA (100 nM). RhoA, phosphorylated ERM (p~ERM, index of RhoA/ROCK pathway activation), Arg-2, total eNOS, eNOS phosphorylated at Ser1177 (pSer1177~eNOS, activated eNOS) and pThr495~eNOS (inactivated eNOS) were determined by Western blot. Results. There were no changes in the levels of eNOS nor RhoA protein, but increased levels of pThr495~eNOS along with reduced pSer1177~eNOS were observed in HUVEC exposed to hypoxia (24 hours). Furthermore, hypoxia was related with increased (~ 2 fold) Arg-2 expression. These effects were not affected by ROCK inhibition or RhoA knockdown, despite that RhoA/ROCK signalling pathway activation (p~ERM) was increased by hypoxia. However, the hypoxia-induced arginase activity was partially blocked by ROCK inhibition and RhoA silencing. Conclusions. RhoA/ROCK signalling pathway is not involved in the changes in Arg-2 expression and eNOS activation in HUVEC exposed to hypoxia. However, it remains to be determined whether RhoA/ROCK activation participates in the relocalization eNOS and Arg-2 and the increased activity of the latter that takes place in hypoxia.
Supported by FONDECYT 1080534 & 1110977, CONICYT ACT-73 (PIA) & AT-24090200, AT-2410017, Chile. C.P. & B.K hold a CONICYT-PhD fellowship.

F-101

Correlation of Fetal Base Deficit (BD) and Lactate Changes with Severity of Variable Heart Rate (FHR) Decelerations. Michael G Ross,[1] Marquis Jessie,[1] Kevin Amaya,[1] Brad Matushewski,[2] Martin G Frasch,[2,3] Bryan S Richardson.[2] *[1]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Ob/Gyn, Univ. Western Ontario, London, ON, Canada; [3]Ob/Gyn, CHU Ste-Justine Research Center, Univ. de Montreal, QC, Canada.*
Objective: Variable decelerations resulting from umbilical cord occlusion (UCO) are the most common FHR abnormality in labor. Recent guidelines classify variable decelerations without detail as to degree of depth. We hypothesized that variable deceleration severity is highly correlated with fetal BD and lactate accumulation.
Methods: Ovine fetuses (N=7, 124±1 d) were chronically prepared with fetal brachial artery catheters and an inflatable cuff for umbilical cord occlusion (UCO). Following 4 d post-op recovery, animals underwent graded UCOs for one min every 2.5 min with one hour of mild partial UCOs (~30bpm decels), one hour of moderate partial UCOs (~60bpm decels), and then one to two hours of severe complete UCOs (~90bpm decels) until fetal pH reached 7.00, when UCOs were stopped. Fetal arterial blood samples were drawn at baseline, at 20, 40 and 60 min of each UCO hour and at 60 min of recovery. BD changes in response to variable deceleration degree were calculated, assuming the rate of BD clearance between decelerations was equivalent to that during recovery.
Results: Repetitive fetal UCO as studied resulted in development of marked acidosis (pH 7.36±0.01 to 6.99±0.01; BD 1.2±0.7 to -14.6±0.3 mmol/l). Mild, moderate and severe variable decelerations increased fetal BD (0.21±0.03, 0.27±0.03, and 0.54±0.09 per min) and lactate (0.08±0.03, 0.14±0.04, and 0.23±0.07 per min) in direct proportion to severity. During recovery, fetal BD cleared at 0.12 mmol/l per min.
Conclusion: Fetuses can tolerate repetitive mild and moderate variable decelerations with minimal change in BD and lactate. In contrast, repetitive severe variable decelerations may result in marked BD increases, dependent upon frequency. Modified guideline differentiation of mild/moderate versus severe variable decelerations can aid in the interpretation of FHR tracings and optimization of clinical management paradigms.

F-102

Deterioration in Fetal Lamb Blood Gas and Acid-Base Status with Advancing Gestation. Dan W Rurak, KS Jospeph. *Obstetrics & Gynecology, University of British Columbia, Vancouver, BC, Canada.*
The fetuses-at-risk approach shows that stillbirth rates increase with advancing gestational age (GA) (1). A causal mechanism may lie in the progressive decreases in weight normalized umbilical blood flow (2) and fetal vascular Po_2 (3) in the human, perhaps due to the rapid fetal 3rd trimester growth, leading to the fetus outstripping the ability of the placenta to provide it with oxygen and other nutrients. Whether this limitation exists in other species is less clear. In the fetal lamb, there is also a decrease in weight normalized umbilical blood flow with advancing GA (4) and higher blood O_2 saturation at mid compared to late gestation (5). However the changes in other fetal lamb blood gas variables in relation to GA have not been determined. Thus the objective of the current study was to assess to relationship between GA and arterial Po_2, Pco_2, pH, base excess (BE), O_2 saturation (So_2), hemoglobin (Hb), O_2 content (Co_2) and glucose (G) and lactate (L) concentrations in chronically instrumented fetal lambs (n=113), that delivered alive at term or preterm. The daily fetal blood samples used (n = 447) were collected under control conditions between 103 and 146 d gestation. The relationships between GA and the measured variables were determined using the least squared method. The results are presented in the Table and include for each variable the regression coefficient ± SE, p value, and the calculated values at 100 and 147 d.

Variable	Slope ± se (p)	100 d	147 d
Po2 (mm Hg)	-0.119 ± 0.036 (<.001)	27.1	21.5
Pco2 (mm Hg	0.123 ± 0.032 (<.001)	44.0	49.5
pH	-0.0155 ± 0.0003 (<.001	7.390	7.321
BE (meqiv/L)	-0.054 ± 0.019 (<.01)	2.6	0.0
So2 (%)	-0.922 ± 0.102 (<.001)	81.8	38.5
Hb (g%)	0.065 ± 0.011 (<.001)	8.7	11.7
Co2 (mM)	-0.037 ± 0.007 (<.001)	4.7	2.9

There are significant decreases in Po_2, pH, BE, So_2, and Co_2 and increases in Pco_2 and Hb. There were no significant changes in G and L concentrations. Since there is no evidence for an increase in fetal cardiac output with advancing GA, it is concluded that in sheep, as in the human, O_2 delivery to fetal tissues decreases with advancing GA. As a consequence, the risk of stillbirth with advancing GA that is present in human pregnancy may also be present in sheep and other species. 1. Joseph KS. BMC Pregnancy Childbirth. 28:7:4, 2007. 2. Sutton MG et al. Cardiovasc Res 25:603-8, 1991. 3. Soothill PW et al. Fetal Ther 1:168-75, 1986. 4. Hedriana HL et al. J Soc Gynecol Investig 2:727-34, 1995. 5. Bell AW et al. Am J Physiol 250:E538-44, 1986.

F-103

The Syncytiotrophoblast (ST) Volume Is Critical for Human, but Not Non-Human Primates (NHP) Stillbirths (SB) at Term. N Schlabritz-Loutsevitch,[1] J Samson,[1] M Schenone,[1] G Mari,[1] G Hubbard,[2] E Dick,[3] Ch Dudley,[4] D Dudley.[4] *[1]Obstetrics and Gynecology, University of Tennessee Health Science Center; [2]Pathology, University of Texas Health Science Center at San Antonio; [3]Pathology, Texas Biomedical Research Institute; [4]Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio.*

Background: SB is a serious obstetric problem with an incidence of 0.05-10% of all pregnancies, however up to 50% have no known cause. The function of the placenta (Pl) is critical for *in utero* life. NHP are the excellent models for both Pl and SB research. The comparative approach is a powerful tool for the development of interventional strategies. Despite the intensive research in Pl pathology, there are no studies available regarding Pl volumetric composition in SB. Fetal hypoxia is associated with the descreased Pl vascularization. We hypothesize that Pl vascularization will be reduced in human and NHP SB as compared to live birth (LB). **Objective:** to evaluate the volumetric Pl composition (ST and villous volumes, volume of fetal capillaries in the terminal, intermediate and stem villi) in humans and NHP SB. **Material and Methods:** 10 Pl from uncomplicated LB and 12 placentas from SB at 37-43 weeks of human pregnancy were collected. Additionally 3 LB and 6 SB term baboons Pl were evaluated. BX 61 Olympus microscope with Computer Assisted Stereology Toolbox software was used for calculation of Pl volumetric composition (Placenta 28 (8-9): 783-93). Statistical analyses were performed using a two-sample student's t test if data met parametric assumptions. If assumptions were not met, two sample Wilcoxon-Mann-Whitney Rank Sum test was used. Significance was set at $p<0.05$. **Results:** The total fetal capillary volume in SB (14.43 ± 3.2 ml) was lower compared to LB (95.96 ± 19.7 ml) in humans and in baboons (5.3 ± 1.0 ml in SB vs 10.18 ± 0.7 ml in LB). The ST volume was lower in SB (59.44 ± 13.48ml) compared to LB (98.27 ± 16.75ml) in humans, but not in the baboons. There were no differences in villous tree volume. **Conclusion:** Decreased ST volume has been described in Intrauterine Growth Restriction in human pregnancy and is associated with the extensive trophoblast shedding. The absence of decreased ST volume in NHP could be explained by the absence of shedding phenomenon, alternatively if shedding occurs, pregnancy loss due to this effect may occur prior to term. ST apoptotic pathway may be a target for SB prevention.

F-104

Functional Development of Fetal SheepVascular Resistance Vessels. S Rupprecht,[1] J Mueller,[1] S Franke,[1] H Schubert,[1] Mark J Nijland,[2] Peter W Nathanielsz,[2] Matthias Schwab.[1] *[1]Hans Berger Department of Neurology, Friedrich Schiller University, Jena, Germany; [2]Center for Pregnancy and Newborn Research, University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

OBJECTIVE; Peripheral resistance (R) artery reactivity plays a major role in blood pressure (BP) regulation. Knowledge of vascular R ontogeny to identify mechanisms and critical periods for fetal programming of vascular disease is limited.

METHODS: We examined fetal sheep mesenteric R arteries with myography at 0.7 (gestation-G; n=6) and 0.9 G (n=6) a critical period in maturation.

RESULTS: Non-receptor mediated muscular vasoconstriction (V/C - Cmax) to KCl (p≤0.003) and receptor mediated V/C (Cmax) to norepinephrine (NE, p≤ 0.007) and endothelin 1 (ET1, p≤0.046) increased at 0.7 to 0.9G. Endothelial (endo) mediated vasodilatation (V/D, DILmax) to acetylcholine (ACh, p≤0.007) increased (Table.1). Max non-receptor mediated non-endo V/D (DILmax) to NO donor sodium nitroprusside (SNP) present by 0.7G remained unchanged (p≤0.786). Vascular sensitivity (EC50) to SNP increased in tendency (p≤0.08) from 0.7 to 0.9G.

CONCLUSIONS: Major non-endo and endo-dependent fetal R artery function matures at 0.7 to 0.9G. Increase in non-receptor and receptor mediated V/C indicates vascular smooth muscle system maturation paralleling the increase in vascular tone and peripheral vascular R, contributing to the term fetal BP rise (Unno, AJP 1999). Increase in receptor mediated endo-dependent vasoreactivity indicates functional endo maturation at 0.7 to 0.9G. While non-endo NO mediated maximum V/D is reached by 0.7G, NO sensitivity increases until 0.9 G indicating the NO pathway matures before other vascular tone regulating pathways.

Table 1 Vascular response in fetal mesenteric arteries at 0.67 and 0.87 gestation

		C_{max}			EC_{50}	
	100dGA	130 dGA	p	100dGA	130 dGA	p
KCl	52.75±6.76	116.56±15.53	0.003	36.79±4.72	22.09±5.19	0.092
NE	69.86±16.76	151.55±9.12	0.007	$1.45E^{-5}$±4.25^{-5}	$1.07E^{-5}$±7.25^{-5}	0.610
ET 1	79.21±9.95	128.05±8.36	0.046	$2.1E^{-8}$±$8.4E^{-9}$	$7.3E^{-9}$±$1.2E^{-9}$	0.410
		DIL_{max}			EC_{50}	
ACh	81.47±9.27	28.73±9.55	0.007	$5.22E^{-7}$±$2.59E^{-7}$	$5.44E^{-6}$±$5.22E^{-6}$	1.00
SNP	18.31±5.06	11.26±6.45	0.786	$2.66E^{-6}$±$2.2E^{-6}$	$1.12E^{-7}$±$7.94E^{-8}$	0.086

C_{max} maximal vasoconstrictive response, DILmax maximal vasodilative response given in percentage of maximal vascular response to 125 mmol/l KCl and percentage to precontraction with 5 µmol NE, respectively. EC_{40} half maximal effective concentration given in mol/l. All values are given as mean±SEM.

F-105

Maternal Mental Stress during Early and Midgestation Accelerates Maturation of Fetal Sheep Resistance Vessel Function. S Rupprecht,[1] J Mueller,[1] S Franke,[1] F Rakers,[1] V Frauendorf,[1] H Schubert,[1] Mark J Nijland,[2] Peter W Nathanielsz,[2] Matthias Schwab.[1] *[1]Hans Berger Department of Neurology, Friedrich Schiller University, Jena, Germany; [2]Center for Pregnancy and Newborn Research, University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Objectives: 30% women experience chronic mental stress in pregnancy (Tegethoff, Environ Health Perspect 2011). Prenatal stress programs vascular dysfunction and hypertension. There are no data on maternal mental stress effects on fetal vascular maturation.

Aim: To examine effects of chronic maternal stress in early and mid-gestation on development of fetal sheep resistance (R) artery function.

Methods: Ewes were isolation stressed between 0.2 and 0.65 gestation (G), 3h twice weekly. Fetal mesenteric R artery function was examined by myography in stressed (0.67 G) and unstressed animals(0.67 and 0.87 G; 6 per group).

Results: Prenatal Stress increased receptor mediated vasoconstriction (Cmax) to ET1, p≤0.02), receptor mediated endothelial vasodilatation (DILmax) to ACh, p≤0.005) and sensitivity (EC50) to the NO donor sodium nitroprusside (SNP, p ≤ 0.089) in tendency at 0.67G. These vascular responses were similar to unstressed fetuses at 0.85G (Table 1). Prenatal Stress did not influence vascular response to KCl, norepinephrine (NE) and PGE2.

Conclusions: Chronic maternal mental stress in early and midgestation accelerates fetal R arteries maturation with specific effects on endothelial function and ET1 and NO mediated smooth muscle vasoreactivity.

Table 1 Vascular response of fetal mesenteric arteries in stressed and unstressed fetuses.

		C_{max}			EC_{50}	
	0.67 gestation controls	0.87 gestation controls	0.67 gestation stress	0.67 gestation controls	0.87 gestation controls	0.67 gestation stress
KCl	52.7±6.8	116.7±15.5	81.7±9.8	36.8±4.7	22.1±5.2	34.4±3.7
NE	69.9±16.8	151.6±9.1	23.7±2.6	$1.5E^{-5}$±$4.3E^{-6}$	$1.1E$±$7.3E^{-6}$	$1.4E^{-5}$±$6.1E^{-6}$
ET1	79.2±1.0	128.1±8.4	135.2±16.8*	$2.1E^{-8}$±$8.4E^{-9}$	$7.3E$±$1.2E^{-9}$	$1.7E^{-8}$±$8.1E^{-9}$
		DIL_{max}			EC_{50}	
ACh	81.5±9.3	28.7±9.6	26.0±10.1*	$5.2E^{-7}$±$2.6E^{-7}$	$5.44E^{-6}$±$5.22E^{-6}$	$2.1E^{-7}$±$1.8E^{-7}$
SNP	18.3±5.1	11.3±6.7	23.1±6.5	$2.7E^{-6}$±$2.2E^{-6}$	$1.1E^{-7}$±$7.9E^{-8}$	$4.1E^{-7}$±$3.0E^{-7}$
PGE_2	90.1±4.6	40.8±20.7	83.9±10.7	$1.7E^{-11}$±$1.7E^{-11}$	$8.2E^{-13}$±$6.5E^{-13}$	$1.1E^{-13}$±$7.3E^{-14}$

C_{max} max vasoconstrictive response, DIL_{max} max vasodilator response in percent max vascular response to 125 mmol/l KCl. and percent to precontraction with 5 µmol NE respectively. EC_{50}half max effective concentration in mol/l. M ±SEM, * p≤ 0.05 vs 0.67G controls.

Friday

F-106

Prenatal Hypoxia as a Second-Insult in Mouse Models of Preeclampsia. Joanna L Stanley,[1,2] Christian F Rueda-Clausen,[1] Dana F Thambiraj,[1] Rajan Poudel,[1] Colin P Sibley,[2] Sandra T Davidge,[1] Philip N Baker.[1,2] *[1]Obstetrics/ Gynecology and Physiology, University of Alberta, Edmonton, AB, Canada; [2]Maternal and Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Background: Mice lacking enzymes such as nitric oxide synthase (eNOS-/-) or catechol-O-methyl transferase (COMT-/-) display a subtle preeclampsia-like phenotype during pregnancy; including hypertension, fetal growth restriction (FGR) and proteinuria (1,2) We hypothesized that a prenatal hypoxic insult would accentuate the differences between controls and transgenic mice and result in a more severe preeclampsia-like phenotype.

Methods: Pregnant eNOS-/-, COMT-/- and control (C57Bl6/J) mice were randomized to prenatal hypoxia (10.5% O2) or normal conditions (20.9% O2) from gestational day 10.5-18.5. At day 18.5 blood pressure (BP), proteinuria, fetal and placental morphometry were measured and placentas were collected to measure superoxide and peroxynitrite production, eNOS and iNOS expression.

Results: Prenatal hypoxia caused an increase in BP in all genotypes (average increase in SBP 12 mmHg). Proteinuria was significantly increased ($p<0.05$) in C57Bl6/J (2 fold) and eNOS-/- (3 fold) but not in COMT-/- mice exposed to hypoxia. Fetal survival following maternal exposure to hypoxia differed depending on genotype (C57Bl6/J: 24%, COMT-/-: 52% and eNOS-/-: 9%; ANOVA $p<0.05$) compared to normoxic litters (95±2%). Birth weight was decreased in both C57Bl6/J and COMT-/- mice (36% and 20% decrease compared to genotype control, $p<0.05$) but could not be evaluated in eNOS-/- due to low survival rates. No significant differences in placental levels of superoxide were observed amongst experimental groups ($p=0.54$). Placental levels of peroxynitrite, however, were increased following hypoxia in COMT-/- (6 fold; $p<0.05$), but not C57Bl6/J mice ($p=0.40$). No significant differences in placental levels of eNOS or iNOS were observed amongst experimental groups.

Conclusion: Despite similar effects on blood pressure and proteinuria, eNOS-/- embryos have a decreased tolerance to prenatal hypoxia. Compared to C57Bl6/J, COMT-/- mice exhibited less severe changes in proteinuria and FGR when exposed to prenatal hypoxia. This relative resistance to prenatal hypoxia was associated with a significant increase in placental levels of peroxynitrite.

1 Stanley et al. 2011 Repro Sci 18(4) O-082
2 Kanasaki et al. 2008 Nature 453: 1117-21

F-107

Divergent Effects of Hypoxia on Fetoplacental Endothelial Cell Function. Emily J Su, Hong Xin, John Coon, Serdar E Bulun. *Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Background: Severe fetal growth restriction (FGR) results in reduced fetoplacental blood flow, and decreased flow further exacerbates fetal hypoxemia. Although hypoxia leads to vasoconstriction in most vascular beds, the fetoplacental bed is unique, and the exact mechanisms by which increased fetoplacental vascular resistance occurs in the setting of FGR and relative fetal hypoxia remain incompletely understood. Our objective was to determine the effects of hypoxia on fetoplacental endothelial cell function as determined by regulation of vasoactive mediators.

Methods: Primarily isolated human fetoplacental endothelial cells were subjected to culture under hypoxic conditions (1.5% O2/5% CO2/bal N2) or normoxia for varying time points. Real-time PCR and western blotting were performed. Hypoxic effects on endothelial cell mediators of vasomotor tone including COX-1 and -2, prostacyclin synthase (PTGIS), prostaglandin E synthase (PTGES2), prostaglandin F synthase (AKR1C3), endothelial nitric oxide synthase (NOS3), and endothelin-1 (EDN1) were assessed.

Results: Cells subjected to hypoxia were exposed to appropriate hypoxic conditions, as demonstrated by induction of vascular endothelial growth factor (VEGFA) mRNA ($p<0.05$) and hypoxia-inducible factor-1α protein ($p<0.005$). HIF-1β expression remained stable with either normoxia or hypoxia. Hypoxia consistently abrogated NOS3 mRNA expression by about one-half ($p<0.05$) and increased EDN1 expression two-fold ($p<0.01$) at both 8- and 24-hours. There was no effect of hypoxia on COX-1, COX-2, or AKR1C3. In contrast, hypoxia initially led to a transient downregulation of PTGES2 at 8 hours ($p<0.05$), which resolved by 24 hours, while hypoxia also significantly induced PTGIS expression at 24 hours ($p<0.0001$).

Conclusions: Hypoxia results in differential regulation of various vasoactive mediators in fetoplacental endothelium. While decreased NOS and increased EDN1 expression levels suggest that hypoxia leads to endothelial dysfunction and vasoconstriction, there is also a significant increase in PTGIS expression. This suggests that the role of hypoxia on fetoplacental vasculature differentially affects various pathways of endothelial cell function. Future studies are required to delineate the effects of hypoxia on fetoplacental endothelial cell function if we are to better understand placental pathophysiology leading to growth restriction and their potential effects on short- and long-term outcome.

F-108

Long-Term Hypoxia Does Not Alter Co-Localization of Heat Shock Protein 90 or Caveolin-1 with eNOS in the Ovine Fetal Adrenal. H Tanaka,[1] KM Kaushal,[1] SM Wilson,[1] DA Myers,[2] CA Ducsay.[1] *[1]Ctr. Perinatal Biology, Loma Linda Univ. School of Medicine, Loma Linda, CA; [2]OB/GYN, Oklahoma Univ. HSC, Oklahoma City, OK.*

BACKGROUND: Endothelial nitric oxide synthase (eNOS) is a key enzyme regulating nitric oxide (NO) production and NO inhibits steroidogenesis. We previously demonstrated in the ovine fetus that long-term hypoxia (LTH) enhances eNOS expression in the adrenal cortex and that NO plays a role in regulating fetal cortisol biosynthesis. However, the activity of eNOS depends not only on the overall expression, but the specific association of eNOS with heat shock protein 90 (Hsp90) and caveolin 1 (Cav-1). In this study, we tested the hypothesis that LTH alters the co-localization of eNOS with Hsp90 and Cav-1 in the ovine fetal adrenal cortex.

MATERIALS AND METHODS: Pregnant sheep were maintained at high altitude (3,820 m) from day 40 of gestation to near term (term = 146 days). Between days 138-141, fetal adrenal glands were collected from LTH and age-matched normoxic control fetuses (n=8-11 per group), sectioned and co-localization of immunoreactive eNOS and Hsp-90 or eNOS and Cav-1 was determined using confocal microscopy. Co-localization of CYP17 with eNOS was also determined. The Manders M1 and M2 coefficients of interpretation were used for quantification of colocalization. All values represent mean±S.E.M.

RESULTS: eNOS co-localized with both Hsp90 and Cav-1 in the fetal adrenal cortex. However, no differences in the degree of co-localization were noted between the control and LTH groups. A high degree of co-localization of CYP17 with eNOS was also observed in both groups.

	Hsp-90/eNOS		Cav-1/eNOS		CYP17/eNOS	
	M1	M2	M1	M2	M1	M2
Control	0.79±0.03	0.86±0.02	0.78±0.04	0.83±0.03	0.74±0.03	0.82±0.02
LTH	0.77±0.07	0.83±0.07	0.81±0.01	0.86±0.01	0.66±0.03	0.79±0.01

CONCLUSIONS: Although there was significant co-localization of both Hsp90 and Cav-1 with eNOS in both control and LTH adrenals, LTH did not affect the degree of co-localization. The high level of co-localization of CYP17 with eNOS strengthens the concept that the adrenal cortical cells themselves are a source of eNOS. We previously showed that under basal conditions, LTH enhances adrenal eNOS activity compared to normoxic controls. However, data from the present study do not support the hypothesis that LTH alters the association of eNOS with the key regulatory proteins Hsp90 or Cav-1 and suggest that the effect of LTH is mediated primarily through enhanced eNOS expression. NIH grant HD31226 and NSF MRI-DBI 0923559

F-109

The Placenta Increases Its Erythropoietin Synthesis during Fetal Hypoxia in Diabetic Pregnancies. Kari Teramo,[1] Mikko Loukovaara,[1] Vedran Stefanovic,[1] Esa Hamalainen,[2] Sture Andersson.[3] *[1]Dept. of Ob/Gyn, University Central Hospital, Helsinki, Finland; [2]Dept. of Clinical Chemistry, University Central Hospital, Helsinki, Finland; [3]Dept. of Pediatrics, University Central Hospital, Helsinki, Finland.*

Background: Hypoxia is the main stimulus of erythropoietin (EPO) synthesis in the fetus. EPO does not cross the placenta, it is not stored and hence fetal serum concentration of EPO is determined by the rate of fetal EPO synthesis and elimination. In the fetal lamb the placenta becomes the major synthesis site of EPO during hypoxia (Davis et al. AJOG 2003;189:1764). The aim of the present study was to measure serum EPO concentrations in umbilical vein (UV) and artery (UA) at birth in diabetic pregnancies, and use the UV/UA EPO ratio as an indication of predominant fetal or placental EPO synthesis.

Methods: 33 type 1 diabetes mellitus (DM) and 3 insulin-treated gestational DM women were studied. 31 patients were delivered by cesarean section before labor contractions, 5 had a vaginal delivery. The umbilical cord was doubly clamped at delivery. pH and blood gases were measured in the UA. Serum samples were obtained separately from the UA and UV. Serum EPO concentrations were measured by a chemiluminescent immunometric assay (Immulite 2000® Siemens). EPO concentrations were log transformed before statistical analyses.

Results: Median maternal prepregnancy BMI was 24.0 kg/m^2 (range 18.8-44.2). Median last HbA_{1c} before delivery was 7.2% (5.9-9.1), median gestational age 254 days (236-275) and median birth weight (BW) z-score +2.9 SD (-2.4 - +5.5). 23 newborn infants were macrosomic (BW z-score >+2.0 SD). Median UA pH was 7.24 (7.07-7.36) and UA pO_2 2.2 kPa (0.6-3.2 kPa). Median UA serum EPO was 94.3 mU/ml (7.4 – 3190) and serum UV EPO 93.4 mU/ml (6.1-3420). UA EPO correlated negatively with UA pH and pO_2 (r=-0.45, p=0.006 and r=-0.53, p=0.0009, respectively). The UV/UA EPO concentration ratio was >1.0 in 21 diabetic pregnancies. The UV/UA EPO concentration ratio correlated negatively with UA pO_2 (r=-0.39, p=0.019), but not with UA pH.
Conclusions: The negative correlation of UV/UA serum EPO ratio with UA pO_2 at birth is the first observation in human pregnancy. It suggests that the placenta becomes the predominant site of EPO synthesis during fetal hypoxia in diabetic pregnancies. We speculate that the fetus increases its EPO synthesis in order to protect its brain and other vital organs during hypoxia.

F-110

Chronic Hypoxia Differentially Attenuates PKG-Mediated Inhibition of Contraction in Fetal and Adult Ovine Carotids. Richard B Thorpe, James M Williams, William J Pearce. *Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*
OBJECTIVE: Chronic hypoxia alters arterial structure and function and produces age-specific changes in vasorelaxant responses to nitric oxide. We recently observed that chronic hypoxia also downregulates the ability of Protein Kinase G (PKG) to inhibit contractility in an age-dependent manner. The present study examines the hypothesis that chronic hypoxia depresses the activity of PKG toward multiple protein targets in the 5-HT induced excitation-contraction pathway more in adult than fetal arteries.
METHODS: Adult and fetal sheep were acclimatized at sea level or at altitude (3280m) for the last 110 days of gestation. Carotid arteries from term fetuses and non-pregnant adults were mounted in vitro for contractility studies. Concentration-response relations for 5-HT were used to quantify changes in 5-HT receptor binding affinity and occupancy using the Furchgott method of partial irreversible blockade. The relative influences of PKG activation and BK channel inhibition on 5-HT induced contractions were determined in the presence and absence of the PKG activator 8-pCPT-cGMP and/or the BK channel inhibitor iberiotoxin. Expression and activity of PKG-I isoforms were determined by Western blot and enzyme activity assay.
RESULTS: PKG activation attenuated the maximum 5-HT efficacy ~64% in adult normoxic (AN), but <3% in adult hypoxic (AH) arteries. Fetal values averaged 36% in normoxic (FN) and 18% in hypoxic (FH) arteries. PKG activation similarly reduced binding affinity by ~ 0.5 log units in both AN and FN arteries, but these effects were absent in hypoxic arteries. Hypoxia had no effect on total PKG abundance or catalytic activity in either fetal or adult arteries, but both abundance and activity were greater in fetal than adult arteries. Hypoxia also had no effect on the BK channel-independent component of PKG-induced vasorelaxation, but virtually eliminated the BK channel dependent component in hypoxic arteries.
CONCLUSIONS: Chronic hypoxia appears to reduce NO-mediated vasorelaxation of 5-HT contractions through depression of ligand binding affinity, and through elimination of the ability of PKG to activate BK-channel mediated vasorelaxation. The age-dependence of this effect appears to arise from the greater abundance and activity of PKG in fetal compared to adult arteries, but could also involve age-related differences in BK channel abundance and regulation.

F-111

HSP27 Expression Is Associated with Improved Right Ventricle Function in Congenital Heart Disease in Children: Links to Placental Insufficiency. Susan Walker,[1] Mark Danton,[2] Edward Peng,[3] Fiona Lyall.[1] *[1]Medical Genetics, Univ. of Glasgow, Glasgow, United Kingdom; [2]Cardiac Surgery, Yorkhil Hospital, Glasgow, United Kingdom; [3]Cardiothoracic Surgery, James Cook University Hospital, Middlesbrough, United Kingdom.*
Introduction:Increases in placental flow impedance, lead to adjustments in growth of the embryonic/fetal heart (1). The developing heart alters its growth to maintain stroke volume to accommodate changing vascular conditions. Severe placental insufficiency can lead to increased loading of the right ventricle (RV). How this may link to development of, and changes that occur in, congenital heart disease is not known but both involve ischemic injury. RV dysfunction occurs after surgical repair of Tetrallogy of Fallot (TOF). A possible cause may be the myocardium of cyanotic patients being more susceptible to ischemic-reperfusion injury during cardiopulmonary bypass. HSP27 is a heat shock protein, the expression of which increases following cell stress and provides a protective role.
We examined the expression of HSP27 in myocardium resected from TOF patients undergoing corrective surgery and hypothesised it would be related to RV function and clinical outcome.
Methods: 10 cyanotic (C) and 10 non-cyanotic (NC) TOF patients were studied. Biventricular function was quantified by Tissue Doppler ECG and compared with 15 matched healthy children. Post-operative systemic perfusion was assessed by mixed venous O_2 saturation (SvO_2), O_2 extraction ratio (OER) and lactate. Western blotting was used to quantify expression of HSP27 from resected RV outflow tract myocardium.
Results: In the C group HSP27 expression in tissue collected during the first 15 min of aortic cross clamp correlated highly with OER (p=0.028). This relationship was not seen in the NC group (p=0.34). In the C group baseline HSP27 expression significantly correlated with post-operative basal septal velocity (p=0.036) but not in the NC group (p=0.93). There was no correlation of HSP27 with tricupsid or mitral annular velocity. Higher baseline HSP-27 was associated with better $mSvO_2$ in the C group (p=0.02) but not the NC group (p=0.93).
The association of HSP27 expression with improved right ventricle function and systemic perfusion suggests an important cardio-protective effect of HSP27 in cyanotic TOF. Placental insufficiency and cause/consequences of TOF may share common pathways.
1. Thornburg K Placenta 2010:31:S54-9

F-112

Human Electronic Fetal Monitoring (EFM) Classification Applied to Fetal Sheep. P Warrick,[1] E Hamilton,[1] B Richardson,[2] M Frasch.[3] *[1]Medical R&D, PeriGen, Montréal, QC, Canada; [2]ObGyn, UWO, London, ON, Canada; [3]ObGyn, U. de Montreal, Montreal, QC, Canada.*
Introduction: Several graded classification methods exist for intrapartum EFM to detect fetal intolerance to labor and prevent hypoxic injury. The relationship between EFM and acidemia can rarely be studied directly in humans because pH is usually measured only at birth and how acidemia evolves is speculative. Using blood gases taken at birth we have measured the performance of a 5-level classification method (Parer and Ikeda, 2007), in term fetuses using a computer based EFM classification system (Elliot et al, 2010).
In contrast, fetal sheep provide an opportunity to manipulate umbilical blood flow, measure fetal blood gas status and observe EFM changes.
Objective: To measure the Parer detection of low pH in fetal sheep and compare to those obtained previously in humans.
Methods: 11 near-term fetal sheep were subjected to repeated partial umbilical cord occlusion and monitored for heart rate and arterial blood pressure. The occlusion severity was increased from mild to moderate to severe levels for approximately one hour each until the pH reached 7.0. Periodic blood samples provided metabolic data every 20 minutes. The FHR was analyzed by a computerized method (PeriCALM Patterns, Princeton, NJ) to assign a Parer classification at each instant in time.
Results: Fig. 1 shows cumulative distribution functions (CDFs) of Parer levels as a function of pH. Using the more severe orange level as a detector of low pH (< 7.12, selected from the CDFs), generated a sensitivity of 46.0% and false positive rate of 14.5%.
Conclusions: Despite being devised for humans, low-pH detection with the Parer system in sheep fetuses was comparable to our human fetus studies detecting metabolic acidemia (sensitivity 32.1%, false positive rate 13.3%, average pH 7.09).
The results of this study support the inferences we are making on human fetal states regarding the presence of acidemia using such a 5 level classification method. It also confirms the need for better methods to improve sensitivity and specificity.

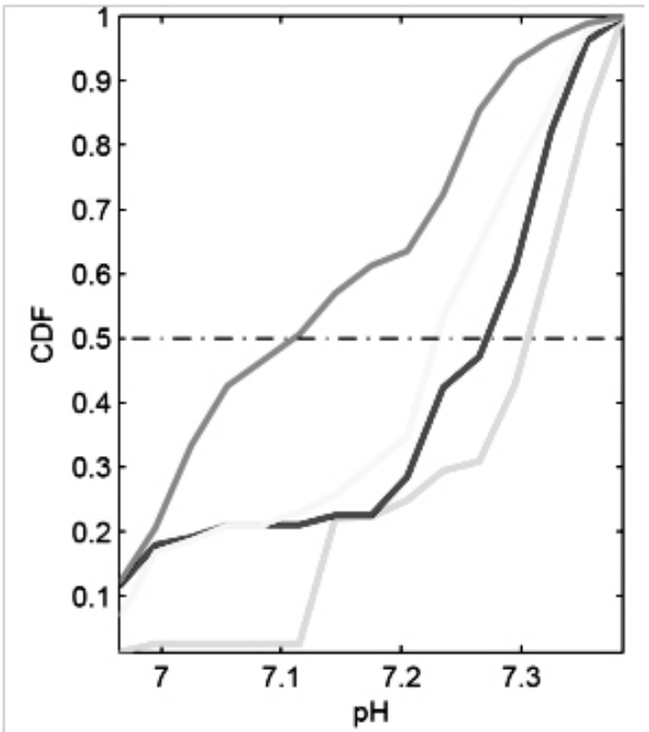


Fig 1: CDFs for 4 Parer levels, equivalent to proportion of time below a given pH.

F-113

Effect of Long-Term High Altitude Hypoxia on Hormonal-Mediated Adaptation in Regulating Actin Polymerization and Vascular Tone of the Uterine Artery in Pregnancy. Xiaohui Huang, DaLiao Xiao, Lubo Zhang. *Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Objective: During pregnancy, chronic hypoxia enhances uterine vascular tone, which is associated with an increased risk of preeclampsia and fetal intrauterine growth restriction. However, the mechanisms underling chronic hypoxia-enhanced uterine arterial tone are not fully understood. The present study tests whether chronic hypoxia up-regulates PKC-mediated vascular actin polymerization, resulting in enhanced uterine vascular tone during pregnancy. **Method:** Uterine arteries were isolated from nonpregnant (NPUA) and pregnant (PUA) (~140 day gestation) sheep maintained at either sea-level or high altitude (3,820m for 110 days, PaO_2: 60mmHg). PKC-mediated vascular actin polymerization and contractions were determined in the 4 groups of sheep. **Results:** Activation of PKC by PDBu produced concentration-dependent contractions in both NPUA and PUA, which were inhibited by an actin polymerization inhibitor cytochalasin B (Cyto B). Long-term high altitude hypoxia significantly increased the PDBu-induced contractions in PUA, which were restored by the inhibition of actin polymerization with Cyto B. In contrast, hypoxia had no effect on PDBu-induced contractions in NPUA. Treatment of NPUA with 17b-estradiol (E_2) and progesterone (P_4) for 48 h attenuated PDBu-induced contractions in normoxic, but not in hypoxic animals. However, E_2 and P_4 did not affect the inhibition of Cyto B in PDBu-induced contractions of NPUA from either normoxic or hypoxic sheep. In normoxic PUA, the MAPK inhibitor, PD098059 significantly potentiated PDBu-induced contractions, but it did not alter the inhibitory effect of Cyto B on PDBu-induced contractions. In hypoxic PUA, PD098059 had no affect either on PDBu-induced contractions or the inhibitory effect of Cyto B. In addition, PDBu produced dose-dependent increases in actin polymerization, which was inhibited by Cyto B but not by PD098059 in PUA. **Conclusions:** Our data suggest that steroid hormones and ERK play a role in pregnancy-mediated attenuation of PKC-induced contractions of the uterine arteries *via* signaling mechanisms upstream of actin polymerization. Chronic hypoxia suppresses the hormonal mediated adaptation in regulating actin polymerization and vascular tone of the uterine artery, which is likely to contribute to the adverse pregnancy outcomes caused by high altitude hypoxia. (Supported by NIH grant HD31226).

F-114

Upregulated Adipocyte Renin-Angiotensin System Contributes to Programmed Hypertension in Offspring of Obese Dams. Cristiane Guberman, Guang Han, Michael G Ross, Mina Desai. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

OBJECTIVE: Obesity accounts for 70% of patients with essential hypertension. Although renal renin-angiotensin system (RAS) is known to regulate blood pressure, it is now recognized that RAS is also activated in adipose tissue during obesity. Angiotensinogen (AGT) is the only known precursor of the vasoactive peptide angiotensin II. Using a rat model, we have previously shown that maternal obesity/high fat (HF) diet results in normal birth weight newborns which develop obesity and hypertension. We hypothesized that adipose tissue RAS is activated during obesity and may be one of the underlying mechanisms contributing to obesity-mediated hypertension in these offspring.

METHODS: At 3 week of age, female rats were weaned to high fat (HF: 60% k/cal) or (control, 10% k/cal) diet. At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy and lactation. After birth at 1 day of age, the litter size was culled to 4 males and 4 females and pups nursed by their own dams. After birth at day 1, and at end of nursing period at 3 weeks of age, subcutaneous adipose tissue was harvested from male offspring. Protein expression (Western Blot) of AGT and angiotensin-converting enzyme (ACE) was determined, values normalized to GAPDH and presented as fold change.

RESULTS: At 1 day of age, despite comparable body weights as the Controls, newborns of HF-fed dams showed increased adipose tissue expression of AGT (2-fold) and ACE (3-fold). At 3 weeks of age, HF offspring were heavier than Controls (68±2 vs 54±2 g). AGT remained elevated in HF offspring (1.3-fold; $p<0.05$) as compared to Controls, though ACE expression was comparable.

CONCLUSION: The offspring of HF-fed dams demonstrated upregulated subcutaneous adipose tissue AGT and ACE at birth, with maintained elevated AGT at 3 weeks. Provided that ACE is not a rate limiting enzyme in adipose tissue, these findings suggest that enhanced adipose tissue RAS may contribute to programmed hypertension in offspring of obese/HF dams.

F-115

Obesity in Male Offspring in Response to Gestational Hypoxia in the Sprague-Dawley (SD) Rat. Sunam Gurung,[1] Roman B Grant,[2] Sarah M Myers,[2] Rheal A Towner,[3] Dean A Myers.[2] *[1]Cell Biol., Univ. Oklahoma HSC, Oklahoma City, OK; [2]OB/GYN, Univ. Oklahoma HSC, Oklahoma City, OK; [3]Adv. Magnetic Res. Cntr., Oklahoma Med. Res. Found., Oklahoma City, OK.*

BACKGROUND: Outbred male SD rats demonstrate a bimodal distribution of obesity on a high fat diet (HFD) exhibiting both diet induced obese (DIO) and DIO resistant (DR) rats. We have previously noted that moderate long term gestational hypoxia increases expression of pro-adipogenic genes in the ovine fetus (PMID:18287225). In the present study we examined the impact of moderate gestational hypoxia in the SD rat on adiposity in male offspring on a high (HFD) or control (CF) fat diet.

METHODS: Pregnant SD rats were subjected to hypoxia (Hyp; 12% O2; n=4) from 15 through 18 days gestation. Normoxic dams (Norm; n=4) served as controls. At birth, litters were culled to 8 pups/dam. At weaning (28d), males were placed on either a HFD (45% kcal fat) or CF (10% kCal fat). At 28 wks, males (n=10/group) were subjected to fMRI analysis of peritoneal (PF) and subcutaneous fat (SCF; expressed as volume; cm²±SEM). Data were analyzed with ANOVA; Tukey's post-hoc test.

RESULTS: There was no effect of gestational hypoxia on birth weights (Norm: 1.64±0.068g vs. Hyp: 1.55±0.089g). At 28 wks post-weaning, body weight (BW) for Norm x CD were normally distributed while Hyp x CD, Norm x HFD and Hyp x HFD exhibited a bimodal distribution of BW. Each group was separated into the upper (U50) and lower (L50) 50%tile for fat analysis. (a: $p<0.05$ vs. Norm x CF)

Norm rats in the upper 50%tile BW had increased PF but not SCF on the HFD; in contrast, Hyp offspring in the upper 50%tile BW exhibited increased PF and SC on both HFD and LFD. There were no differences in adipose deposition between Norm and Hyp in the lower 50%tile BW.

CONCLUSIONS: Gestational hypoxia programs adiposity on a LFD and enhances obesity on a HFD at the level of SC expansion. The genes that confer DIO resistance protect against gestational hypoxia programming of obesity. HD050620

Table1

	Peritoneal Fat			
	CF		HFD	
	Norm	Hyp	Norm	Hyp
U50	9.3±0.4	11.8±0.4a	11.5±0.1a	12.8±0.4a
L50	8.5±0.3	8.3±0.3	8.7±0.3	9.0±0.3

Table2

	Sub. Fat			
	CF		HFD	
	Norm	Hyp	Norm	Hyp
U50	4.3±0.2	5.8±0.4a	5.6±0.8	5.7±0.6
L50	2.9±0.2	3.2±0.3	3.1±0.2	2.6±0.3

F-116

Gestational Hypoxia Alters Expression of Genes Regulating Hepatic Fatty Acid Synthesis in the Sprague-Dawley (SD) Rat. Sunam Gurung,[1] Roman B Grant,[2] Krista Hanson,[2] Dean A Myers.[2] *[1]Cell Biol., Univ. Oklahoma HSC, Oklahoma City, OK; [2]OB/GYN, Univ. Oklahoma HSC, Oklahoma City, OK.*

BACKGROUND: We have developed a model of gestational hypoxia induced programmed obesity in the male SD rat where the offspring exhibit obesity on a low fat diet that is high in carbohydrate. In the present study we examined the impact of moderate gestational hypoxia in the SD rat on hepatic expression of genes regulating fatty acid synthesis in the male offspring at adulthood.

METHODS: Pregnant SD rats were subjected to hypoxia (Hyp; 12% O2; n=4) from gestational day 15 through 18. Normoxic dams (Norm; n=4) served as controls. At birth, litters were culled to 8 pups/dam. At weaning (28d), males were placed on a low fat, high carbohydrate diet (10% kCal fat, 70% kcal carbohydrate 20% kcal protein). At 28 wks (n=10/group) the study was terminated and livers were obtained. qRT-PCR performed for fatty acid synthase (Fsn), the long chain fatty acid elongases (ELOVL) 5 and 6, stearoyl-CoA desaturase 1 (Scd-1), stearoyl response element binding protein 1 (SREBP-1) and the housekeeping gene, cyclophilin (Cyclo). Data are expressed as fg mRNA/50ng RNA±SEM. Data were analyzed with Student's t-test; a: $p<0.05$ vs normoxic control.

RESULTS: Gestational hypoxia resulted in increased expression of SREPB-1, Fas, ELOVL5 and 6 mRNA in the liver of male offspring as adults. Scd-1 as well as Cyclophilin were unchanged.

CONCLUSIONS: Gestational hypoxia results in upregulation of genes governing fatty acid synthesis likely contributing to the programming of obesity in the male offspring. The increased expression of a key transcription factor, SREBP-1 regulating this family of genes provides a target for hypoxic programming of liver dysfunction. HD050620

Table1

	Fas	Scd-1	ELOVL5	ELOVL6	SREBP-1	CYLCO
Norm	2.47±0.3	1.87±0.4	4.8±0.8	6.0±0.7	43.1±5.8	32.8±5
Hyp	5.02±0.3a	1.043±0.3	15.21±4a	15±3.2a	124.8±21a	29.7±3.4

F-117

Alteration in Maternal Diet Induces Metabolic and Epigenetic Dysregulation in Young Female Offspring. Hye J Heo,[1] Jessica Michaels,[1] Howard Slomko,[2] Fabien Delahaye,[1] Ciprian P Gheorghe,[1] Yongmei Zhao,[1] John M Greally,[3] Nir Barzilai,[3] Francine H Einstein.[1] *[1]Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine/Montefiore Medical Center; [2]Pediatrics, Albert Einstein College of Medicine/Montefiore Medical Center; [3]Medicine and Genetics, Albert Einstein College of Medicine/Montefiore Medical Center.*

OBJECTIVE: To characterize the metabolic and genome-wide DNA methylation of young female offspring exposed to maternal western diet and calorie restriction.

STUDY DESIGN: SD dams were fed 1 of 3 diets: standard chow (Con), calorie restricted (pair-fed 50% kcal/d of Con) from gestational day 11 through lactation (CR) or western diet from 3 wks through gestation/lactation (WD). Litters culled (8/lit) and fed standard chow post weaning. At 9wks, female offspring (n=9/gp) hyperinsulinemic (3mU/kg/min) clamps were performed using somatostatin (1.5ug/kg/min) and $3H^3$-glucose tracer to follow glucose flux. Massively parallel sequencing-based HELP assay was used as a discovery platform to examine cytosine methylation levels at >1.65 million loci in the liver (3-4/gp).

RESULTS: WD and CR had lower overall insulin sensitivity (IS) compared to Con based on glucose infusion rate (GIR) (Table 1). CR had lower hepatic IS (greater hepatic glucose production (HGP)) and WD had lower peripheral IS (Rd). While no global methylation differences were seen among groups, significant regional differences were found (Figure 1). Differential methylation was revealed in non-overlapping loci within promoter regions and gene bodies of WD and CR compared to Con (WD 74 v CR 19, $p<0.00001$ vs Con).

CONCLUSION: In utero exposure to maternal WD and CR lead to differences in metabolic and epigenetic dysregulation in young female rats and suggests that the underlying pathophysiology for development of overt disease later in life may be different. Assessment of genome-wide epigenetic alterations may provide insight into the mechanisms responsible for developmental programming.

Hyperinsulinemic Clamps

	GIR (mg/kg/min)	HGP (mg/kg/min)	Rd (mg/kg/min)
Con	13.4±0.7	8.0±0.6	21.6±0.8
CR	8.2±0.9*	14.2±0.7*	22.5±1.1
WD	9.3±1.3*	8.7±1.5	17.5±1.9

*p<0.5 compared to Con-F

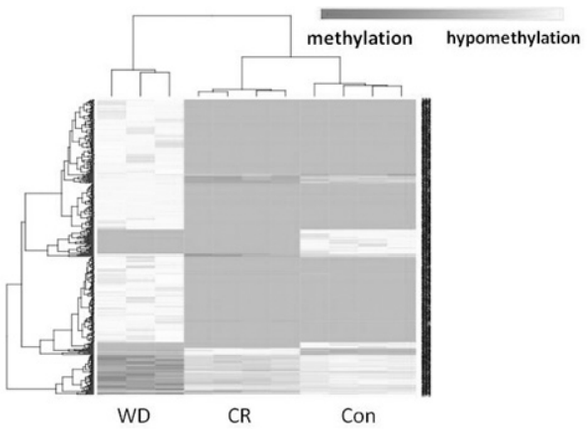


Figure 1. Heatmap of top 500 loci with differences in methylation in female offspring liver
(p value <0.01 compared to controls)

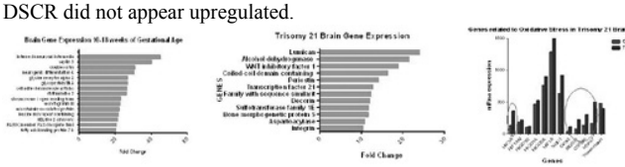
F-118

Human Fetal Brain Development during the First and Second Trimester (FT-ST) as It Compares to Trisomy 21 Fetal Brain Development. Jesus I Iruretagoyena,[1] Wayne Davis,[2] Christina Kendziorski,[3] Cynthia Bird,[1] Rebecca Radu,[1] Aimee Teo Broman,[3] Sandra Splinter Bondurant,[2] Theodore Golos,[1] Dinesh Shah,[1] Ian Bird.[1] *[1]Ob/GYN, U. of Wisconsin; [2]Biotech, U. of Wisconsin; [3]Biostatistics, U. of Wisconsin.*

Objective: To describe brain development in FT and ST human fetuses to create a normal database for comparison with T21.

Study Design: 8 fetal brains 10-18GA prospectively collected at termination of pregnancy. After RNA extraction, samples hybridized into an Affymetrix 1.0 ST chip. The second part consisted in investigating differences within 10-18 GA. Samples were divided by GA into 10-14 and 15-18. The third part consisted in comparing 2 T21 brains from 16-18 GA to age matched controls. qRT PCR in progress. A fold change of 2 or above adjusted for a false discovery rate of 5% used for individual genes. Gene Ontology/KEGG to identify functional groups.

Results: Brains from 10-18 GA showed expression of genes for neuronal migration, differentiation and connectivity. FT brains showed genes coding for neuronal migration upregulated >5 fold vs. ST which showed genes coding for neuronal differentiation/connectivity upregulated >5 fold. ALDH1A1/ NPY marker of spinal cord and striatum were upregulated in FT and ST respectively. SLITRK6-HAS2 and CRYAB-PCDH18 genes for ear and eye sensory input were upregulated in FT. T21 Brains showed upregulation for neuronal migration, oxidative stress and estrogen degradation. Genes at the DSCR did not appear upregulated.



Conclusions: For the first time global gene expression of brain was described in human samples to create a database of normal development to understand and compare with T21.FT is dominated by neuronal migration, differentiation, programmed cell death and sensory organs. ST is dominated by neuronal proliferation, branching and myelination.T21 showed a delay in neuronal maturation, increased oxidative environment and upregulation of genes for lumican-decorin. These and our discovery of increased degradation of estrogen in the T21 brain may explain the neurodevelopmental delay and provide the first evidence that T21 brains show Alzheimer's type changes in the fetal period.

Friday

F-119

Infantile Growth after Undernourishment In Utero Positively Correlates with Chronic Inflammatory Reactions in the White Adipose Tissues of Adult Mice, as a Risk of Developing Obesity. Hiroaki Itoh, Kohmura Yukiko, Keiko Muramatsu, Kaori Yamazaki, Kotomi Nagahashi, Yuki Nakamura, Naoaki Tamura, Toshiyuki Uchida, Kazunao Suzuki, Naohiro Kanayama. *Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka-ken, Japan.*

Objective; The infantile rapid growth after undernourishment *in utero* was reported to be a risk factor of adult obesity. Recently, it was demonstrated that the chronic inflammation, with an increase of small size adipocytes, in the white adipose tissues (WAT) plays a pivotal role in the development of obesity and associated metabolic disorders. In this study, we hypothesized that rapid growth during infantile period after undernourishment *in utero* accelerates chronic inflammation in adult WAT and constitutes a risk of obesity under western life style. To prove the hypothesis, we developed a mouse animal model of undernourishment *in utero* and compared between the growth at weaning and chronic inflammation in WAT after a high fat diet (HFD) at 17wks. ***Methods;*** Maternal caloric restriction (30% reduction l) was apply to C57/BL6 pregnant mice. At 3 wks, body weight distribution of the offspring was assessed by the formula of [(body weight) – (mean body weight)] / standard deviation (SD) of body weight = SD ratio at weaning. After a HFD (60% fat) from 9 to 17 wks, the body weight and weight of subcutaneous WAT, serum levels of glucose and total cholesterol, rate of small adipocyte (less than 30 um in diameter), immunohistochemical detection rate of macrophage specific F4/80 in WAT were assessed. The gene expression of inflammatory M1 macrophage-specific CD11c and anti-inflammatory M2 macrophage-specific CD163 in WAT were measured by quantitative RT-PCR. ***Result;*** At 17 wks, all of the body weight (r=0.85), weight of subcutaneous WAT (r=0.07), serum glucose levels (r=0.67), total cholesterol levels (r=0.46), the rate of small adipocyte (r=0.49), immunohistochemical detection rate of macrophage specific F4/80 (r=0.54), the M1/M2 macrophage ratio of CD11c/CD163 gene expression were positively correlated with SD ration at weaning (3 wks) in undernourished offspring (all P<0.001), but not in normally nourished offspring. ***Conclusion;*** It is suggested that high growth rate during infantile period after undernourishment *in utero* accelerates chronic inflammation in the adult WAT in response to a HFD and contributes, at least partly, to the deterioration of obesit.

F-120

Chronic Intrauterine Hypoxia Programs Hepatic Mitochondrial Enzyme Activity in Guinea Pig (GP) Offspring. Sheveta Jain, Yazan M Al-Hasan, Loren P Thompson. *Dept. of Obstetrics, Gynecology & Reproductive Sciences, Univ. of Maryland School of Medicine, Baltimore, Md.*

Introduction: Intrauterine stress and adverse events during gestation contribute to fetal organ dysfunction. Oxidative stress is emerging as an important prenatal insult that may initiate programming of metabolic disorders in the affected offspring. We reported that chronic hypoxia (HPX) *in-utero* reduces mitochondrial CCO (cytochrome c oxidase) activity in near term fetal GP livers (EB2011, Abstract #851.4). However, it is unclear whether these changes in hepatic mitochondria are sustained in the offspring. Thus, we hypothesized that offspring exposed to prenatal HPX exhibit changes in hepatic enzyme activity associated with fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS). **Methods**: Pregnant GPs were exposed to either air (normoxia, NMX) or 10.5% O_2 (hypoxia, HPX) for 14d prior to term (65d). Food (g/d) and water (ml/d) intake were measured during treatment. Fetal GPs were allowed to deliver and selected male offspring were housed in room air with the mothers until weaning (30d). At 90d, offspring (N=11-12/group) were anesthetized, body and liver weights measured, and right liver lobes were frozen and stored (-80°C). Liver mitochondria were isolated and activity of MCAD [medium chain acyl dehydrogenase, oxidation of ferrocenium (mM/min/mito protein)] and CCO [complex III enzyme, oxidation of cytochrome c (µmoles/min/mito protein)] were assayed in the same samples. **Results**: Chronic HPX had no effect on food intake (38.7±2.5 vs 37.5±1.7, NMX vs HPX) but increased water intake (96.3±10.8 vs 127.7±13.6, p<0.05) of pregnant sows. Birth weights (93.2±4.3 vs 82.2±2.2 NMX vs HPX) were reduced (p<0.05) with HPX but did not differ at 90d. Prenatal HPX increased (p<0.05) both MCAD activity by 18.4% (NMX vs HPX; 0.131±0.005 vs 0.155±0.008) and CCO activity by 77.9% (NMX vs HPX; 2.096±0.188 vs 3.731±0.255) in HPX vs NMX controls. This contrasts to the HPX-induced decrease in fetal liver CCO activity previously reported. **Conclusions**: Chronic prenatal HPX may increase FAO and OXPHOS in livers of male offspring. This effect on hepatic mitochondrial function is unrelated to nutritional deficiency in response to HPX. This study identifies an important programming response of prenatal HPX on hepatic mitochondrial function that may contribute to altered liver metabolism in the offspring. (NIH HL49999)

F-121

HPA Axis Responses to Hypoglycemia in 1 and 2 Year Old Ponies Following Neonatal Cortisol Over-Exposure. JK Jellyman,[1] OA Valenzuela,[1] VL Allen,[1] AJ Forhead,[1] NB Holdstock,[2] AL Fowden.[1] *[1]Physiology, Development and Neuroscience, University of Cambridge; [2]Clinical Veterinary Medicine, University of Cambridge, United Kingdom.*

We have previously shown that over-exposure to cortisol by neonatal ACTH-treatment does not alter basal or ACTH-stimulated cortisol levels in young adult ponies. This study examined whether early-life cortisol over-exposure altered the HPA-axis response to the physiological challenge of hypoglycaemia.

Methods All procedures were carried out under the UK Animals (Scientific Procedures) Act 1986. Foals received either saline (0.9% NaCl, n = 8, control) or long acting ACTH (depot synacthen 0.125mg im 2x daily, n = 7) for 5 days from day 1after birth. At 1 and 2 years of age, arterial and venous catheters were inserted under general anaesthesia. After recovery, the ponies were given a bolus of insulin (i.v. Actrapid human insulin, Novo Nordisk; 0.5U/kg). Blood samples were taken at 10min intervals before and after insulin administration to measure plasma ACTH (RIA, Diasorin) and cortisol (RIA, Siemens) concentrations. Values are mean (±SE). Data were analysed by two-way ANOVA.

Results Cortisol levels were significantly higher in ACTH treated than control foals throughout neonatal treatment (P<0.05). Neonatal cortisol overexposure did not alter basal plasma glucose, ACTH or cortisol values at 1 or 2 years (Table 1). At 60min post insulin, glucose levels were significantly decreased and concentrations of ACTH and cortisol were significantly increased from basal values in all groups (Table 1). The increment in plasma ACTH was significantly higher in cortisol-overexposed than control yearlings and the increments in both ACTH and cortisol were significantly higher in cortisol-overexposed ponies at 2 years of age (Table 1).

Table 1.		1 Year		2 Year	
		Control	Cortisol overexposed	Control	Cortisol overexposed
Glucose mmol/l	Basal	6.12±0.38	6.34±0.37	5.92±0.26	5.99±0.24
	60Min	2.30±0.25[a]	2.12±0.19[a]	2.55±0.19[a]	2.0±0.25[a]
ACTH (pg/ml)	Basal	41.6±6.8	37.6±3.2	51.7±3.7	63.6±11.3
	60Min	99.1±16.0[a]	196.4±59.3[ab]	90.0±23.2[a]	207.4±52.6[ab]
Cortisol (ng/ml)	Basal	59.1±6.5	69.2±8.4	56.7±7.1	50.3±5.7
	60Min	115.7±18.0[a]	113.4±11.3[a]	99.5±14.5[a]	119.1±13.2[ab]

[a] P<0.05 versus baseline; [b] P<0.05 versus saline-treated ponies; Two Way ANOVA with Repeated Measures.

These data suggest that cortisol over-exposure induced neonatally by ACTH treatment may programme HPA axis function leading to increased pituitary responsiveness to hypoglycemia in young adult ponies.

F-122

Maternal Glucocorticoids Program Offspring Vascular Expression of miR- 29c. Omid Khorram,[1] Thomas Magee,[1] William J Pearce.[2] *[1]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, USA.*

Objective: We have recently identified Micro-RNAs (miRNAs) in blood vessels whose expression are programmed by maternal diet (AJP, 298(5), 2010). Of these, miR-29c was of greatest interest as its target genes include components of extracellular matrix. The objective of this study was to determine if in vivo blockade of elevated maternal GC in response to undernutrition could block the effect of MUN on offspring vascular miRNA 29c expression.

Methods: Three groups of pregnant Sprague-Dawley animals were used in this study. Group 1:Control dams fed ad-libitum, Group 2: Maternal Undernutrition (MUN) (50% decrease in calories) from day 10-22 of gestation, Group 3: MUN+Metyrapone (Met) in drinking water (0.5mg/ml) days 11-22 of gestation. 1 day old (P1) and adult 5 month old offspring were sacrificed, and aortas were frozen for extraction of RNA. miR-29c expression was determined by real time RT-PCR. To verify targets of miR-29c in the aorta, P1 aortic explants from control animals were transfected with preMIR 29c and following 48 hours of culture the expression of elastin and collagen (Col) 4A5 mRNA were determined by real time RT-PCR. Fold change in mRNA expression were compared by ANOVA followed by Student-Newman-Keuls test.

Results: MUN increased maternal corticosterone levels and this increase was blocked by metyrapone treatment. In P1 offspring (mixed gender) MUN induced respectively -1.4 fold and -1.7 fold decrease in expression of aorta miR- 29c (P=NS). In adult female offspring there was a -2.7 fold decrease (P=.012) in miR- 29c in the MUN group and a 2.75 fold increase (P=.001) in the Met group mRNA expression. In adult males miR-29c expression in the

MUN groups was 1.3 fold higher (P=NS) whereas in the Met groups it was 5.2 fold higher (P=.002) compared with controls. Up-regulation of miR-29c in aortic explants through transfection of preMIR significantly inhibited the expression of Col4A5 and elastin as expected.
Conclusion: These results demonstrate an important novel mechanism in which elevated maternal glucocorticoids in response to MUN programs offspring vascular expression of miR-29c expression in a gender and developmentally regulated fashion. This miRNA-mediated mechanism might be a means by which the uterine environment programs vascular remodeling in the offspring. Supported by American Heart Association (013948-01) (O.Khorram).

F-123

Periconceptional Undernutrition Differentially Alters Insulin Signalling in Skeletal Muscle in Singleton and Twin Fetal Sheep in Late Gestation. S Lie,[1] JL Morrison,[1] O Wyss,[1] S Zhang,[1] SE Ozanne,[2] IC McMillen.[1] [1]*Sansom Institute for Health Research, University of South Australia, Australia;* [2]*Institute of Metabolic Science, University of Cambridge, United Kingdom.*
Introduction: Maternal undernutrition before and/or during early gestation results in a decrease in total myofiber number in fetal sheep and an increased risk of insulin resistance in postnatal life. The aims of this study were to determine the separate effects of maternal undernutrition during the periconceptional (PCUN) and preimplantation (PIUN) period on factors regulating insulin signalling in skeletal muscle from singleton and twin fetal sheep in late gestation.
Hypothesis: We hypothesised that PCUN and PIUN would result in a decrease in gene and protein expression of the insulin signalling factors in skeletal muscle in singleton and twin fetuses.
Methods: Control ewes were fed 100% metabolisable energy requirement (MER) from -45d to 6d post conception. Ewes in the PCUN group were fed 70% MER from -45d to 6d post conception and ewes in the PIUN group were fed 70% MER for the first 6d post conception only. Quadricep muscle samples from singleton and twin fetuses were collected at 136-138d gestation. Gene and protein expression were analysed using Real Time-PCR and Western blot analyses respectively. Two-way ANOVA was used to determine statistical significance.
Results: In singleton fetuses, the abundance of the PI3K catalytic subunit p110β and phosphoPKCζ was lower in the PCUN group, while PKCζ abundance was lower in both PCUN and PIUN groups. Interestingly, in twin fetuses, IRS1 and PKCζ abundance was increased in both PCUN and PIUN groups, while AKT2 abundance was increased in the PCUN group and GLUT4 abundance was increased in the PIUN group. In contrast to singletons, there was no effect of PCUN and PIUN on p110β and phosphoPKCζ abundance in twins.
Conclusion: The adaptation of the singleton embryo to maternal undernutrition during oocyte and/or embryo development to result in a decrease in insulin signalling may limit metabolic demand in developing muscle in anticipation of a reduced nutrient supply during fetal life. The adaptation of the twin embryo, however, may ensure insulin signalling is maintained in the growth restricted twin fetus. The differential effects of PCUN and PIUN suggest that there are specific factors recruited during oocyte maturation and/or the preimplantation period which act to regulate the translation of the key insulin signalling factors.

F-124

Dexamethasone (DM) Administration in F0 Ovine Pregnancy Eliminates the F2 Offspring (OFF) Postnatal (PN) Plasma Leptin (L) Peak (LP) Increasing Appetite and Weight Gain. Nathan M Long,[1] Peter W Nathanielsz,[2] Stephen P Ford.[1] [1]*Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA;* [2]*Center for Pregnancy and Newborn Research, University of Texas, Health Sciences Center, San Antonio, TX, USA.*
Introduction: Both maternal obesity (MO) and maternal synthetic glucocorticoids (sGC) lead to F1 OFF obesity. The newborn (NB) lamb LP is blunted in MO F1 and F2 OFF (J Phys. 589:1455).
Hypothesis: One third the clinical dexamethazone (DM) dose blunts the plasma LP in F2 NB OFF increasing later life appetite and weight gain.
Methods: F0 ewes ate NRC maintenance diet. On d 103 and 104 gestation, DM ewes received 4 x 2 mg DM i.m, 12 h apart and control (C) saline. Ewes lambed naturally. At 22 ± 4 months of age, F1 ewes were mated to produce F2 OFF. F2 OFF (9 females per group) were bled PND 1-7, 9 -11. At 10 m both C and F2 OFF received an *ad lib* feed trial with intake monitored by Growsafe (GrowSafe, Airdrie, Alberta, Canada) for 12 weeks. Plasma cortisol and L were determined (RIA). Body weight, feed intake, glucose and hormones were analyzed.
Results: DM F2 OFF were smaller than C F2 at birth (5.7 ± 0.3 vs 6.6 ± 0.3 kg) with increased cortisol and blunted post-natal (PN) LP (Fig 1). During the feeding challenge DM F2 OFF ate 10 % more feed gaining 20 % more weight ($P < 0.05$) vs. C F2 OFF. After the trial, DM F2 OFF had greater adiposity (DEXA) vs. C F2 (23.9 ± 1.3 vs 17.6 ± 1.6 % $P < 0.05$).
Conclusions: DM to F0 mothers blunted the F2 OFF PN LP. The elevated PN cortisol in DM F2 OFF is similar to that in F1 OFF of obese ewes in which the PN LP is also blunted. These data support our hypothesis that cortisol blunts the LP increasing appetite, weight gain and adiposity similar to outcomes in MO F1 OFF (J. An. Sci 88:3546). To our knowledge these are the first demonstrations of an altered PN LP in OFF of sGC treated mothers associated with a specific later-life metabolic phenotype. NIH INBRE P20RR016474, and HD 21350.

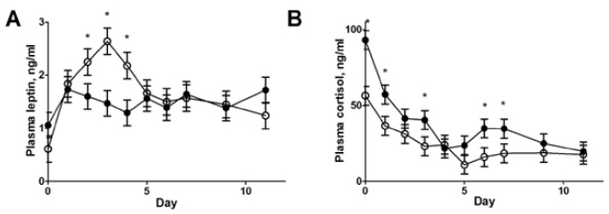


Figure 1. Plasma L (trt x day, *$P < 0.01$) and cortisol (trt x day, *$P < 0.05$) from birth until d 11 in lambs from DM F1mothers (closed) and control F1 mothers (open).

F-125

Maternal Obesity (MO) and Maternal Nutrient Excess (MNE) in Pregnancy Has Adipose Tissue Specific Effects on mRNA for Angiogenic Factors but Not Adipogenic Markers in Late Gestation Fetal Sheep Adipose Tissue (AT). Nathan M Long,[1] Peter W Nathanielsz,[2] Stephen P Ford.[1] [1]*Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA;* [2]*Center for Pregnancy and Newborn Research, University of Texas, Health Sciences Center, San Antonio, TX, USA.*
Introduction: One third of American women of child bearing age are obese (OB). Children of OB mothers are predisposed to obesity. We previously showed that MO and MNE increase fetal AT lipid content and transporter abundance in perirenal (PR) and subcutaneous (SQ) AT.
Hypothesis: MO alters fetal AT depot adipogenic markers, local cortisol availability, and angiogenic factors differentially according to timing of depot development.
Methods: Ewes ate either 100 % (Control; C) or 150 % NRC recommendations (OB) from d 60 before conception until CSection on d 135 gestation - fetuses euthanized by exsanguination under general anesthesia, AT depot weights recorded and tissues frozen in liquid N_2. N = 7 males per group. RNA was extracted from PR and SQ and mRNA expression determined (RT-PCR).
Results: Fetal PR AT (% of fetal carcass weight) was increased in OB vs. C fetuses (0.81±0.01 vs 0.68±0.01; P<0.01) and SQ AT thickness increased (P=0.01) in Ob vs. C fetuses (0.9±0.1 vs 0.5±0.1 mm respectively). Lipid content was increased in PR AT (P<0.05) and tended to increase (P=0.09) in SQ AT of OB vs. C fetuses (913±30 vs 731 ±30 and 115±25 vs 50±25 mg lipid/g AT). Adipogenic and angiogenic gene mRNA in PR and SQ fetal AT at d 135 of gestation are shown in Fig 1.

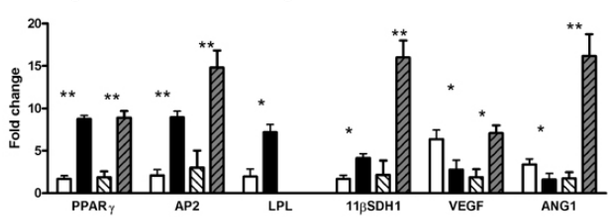


Fig 1. Gene abundance of adipogenic and angiogenic genes in PR (first two and SQ (second pair) day 135 fetal AT from control (light colored bars 1 and 3) and OB (closed and gray bars 2 and 4) mothers. ** means differ P<0.01, * means differ P<0.05
Conclusions: MO increased expression of adipogenic factors (PPAR γ and AP2) and 11βSDH1 (whose activity increases local cortisol in PR and SQ AT. There were depot specific effects for LPL a marker of adipose maturity. Angiogenic factors were decreased in OB vs C fetuses in PR and increased in SQ. NIH INBRE P20RR016474; HD 21350.

Friday

F-126

Prenatal Chronic Hypoxia and Oxidative Stress Programme Insulin Signalling Defects in Adult Rat Offspring. CM Lusby,[1] EJ Camm,[1] MS Martin-Gronert,[2] SE Ozanne,[2] DA Giussani.[1] *[1]Physiology, Development and Neuroscience, University of Cambridge; [2]Institute of Metabolic Science, University of Cambridge, United Kingdom.*

Epidemiological and experimental studies have repeatedly shown that maternal undernutrition programmes the metabolic syndrome in adult offspring (Fernandez-Twinn & Ozanne. *Ann N Y Acad Sci.* 1212:78, 2010). That prenatal chronic hypoxia can also programme metabolic defects in later life is beginning to emerge (Camm et al. *FASEB* **25(1)**:420, 2011; Rueda-Calusen et al. *Diabetes* **60(2)**:507, 2011; Dolinsky et al. *Diabetes* **60(9)**:2274, 2011). However, the mechanisms mediating programming of metabolic dysfunction in hypoxic pregnancy remain elusive. Here, we investigated whether oxidative stress is involved by determining in rats the effects of maternal treatment with vitamin C in control or hypoxic pregnancy on insulin signalling in adult offspring.

METHODS: On day 6 of pregnancy rats were randomised to 4 groups, each n=6: normoxic (21% O2, N) or hypoxic (13% O2, H) pregnancy +/- maternal vitamin C treatment (C; 0.5 g/100ml in drinking water). This model of hypoxia does not affect maternal food intake. At birth, litters were culled to 8 pups. Following weaning, pups were maintained until adulthood. At 3 months, 1 male per litter was subjected to a glucose tolerance test (GTT). Following euthanasia, skeletal muscle from the same male was frozen for Western blot analysis.

RESULTS: At 3 months, offspring of hypoxic relative to normoxic pregnancy had lower serum insulin but similar blood glucose during the GTT (Fig 1.A). Skeletal muscle from offspring of hypoxic pregnancy showed an increase in insulin receptor β (IR β), insulin receptor substrate 1 (IRS-1) and glucose transporter 4 (GLUT4; Fig 1. B+C). These data are indicative of increased insulin sensitivity in young offspring of hypoxic pregnancy. Maternal vitamin C during hypoxic pregnancy prevented these changes from occurring.

CONCLUSION: Chronic prenatal hypoxia and oxidative stress alter insulin signalling in adult offspring and these conditions could have a role in programming metabolic disease in later life.

Supported by The Wellcome Trust and BHF.

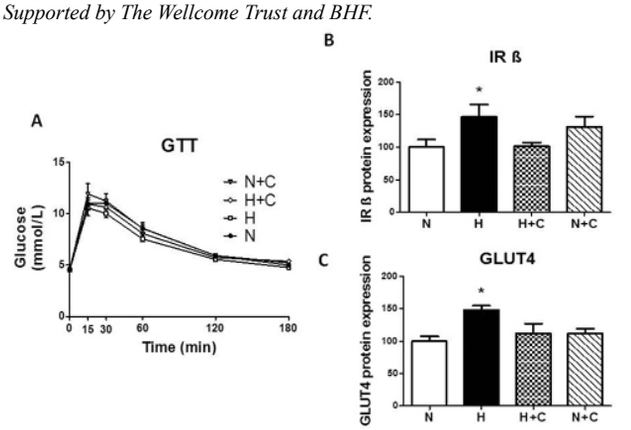


Figure 1 : Mean ± SEM for A: Glucose Tolerance Test; B + C: Expression of insulin receptor β and glucose transporter 4 in vastus muscle; n= 6 for all groups; N = Normoxic, H = Hypoxic, H+C = Hypoxic supplemented with Vitamin C; N+C = Normoxic supplemented with Vitamin C. * vs Normoxic; 2 way ANOVA; p < 0.05; Student-Newman-Keuls test

F-127

Telomere Length in an Adolescent Population: Association with the Duration of Breast-Feeding. Richard Maganga,[1] Julie A Marsh,[1] Stephen J Lye,[2,3] Craig E Pennell.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [3]Obstetrics and Gynaecology, The University of Toronto, Toronto, ON, Canada.*

Early life nutrition has long-term effects on adult health. Telomere shortening is associated with stress and age-related disease, and telomere length (TL) is used as a predictor of biological ageing. However, the associations between TL and perinatal factors, including birth size and early life nutrition, are unknown. At 17 years of age blood samples were collected from 1,266 individuals from the Western Australian Pregnancy (Raine) Cohort. Genomic DNA was extracted from blood and TL was measured using real-time PCR (qPCR). Analyses of log-transformed TL were performed using multivariate linear regression on full-term, singleton births of European descent (N=949). The analyses included birth biometry, birth characteristics, maternal smoking, parental biometry and breast-feeding duration.

Mean weight at birth was above WHO standards by 0.3SD; by age one this had increased to 0.5SD, illustrating a trend towards increasing birth weight and weight gain in early childhood. Parental anthropometry and pregnancy characteristics were similar between male and female offspring. The median duration of breast-feeding was 6 months (IQR 2-11). The distribution of TL was similar between males (median:1.4, IQR:1.0-2.1) and females (median:1.3, IQR:1.0-2.1). Positive associations were detected between adolescent TL and neonatal abdominal circumference (p=0.009) and duration of breast-feeding (p=0.003). However, no associations were detected between TL and parental age, parental BMI, maternal smoking, length of gestation, sex, birth weight z-score, neonatal skinfold thickness and change in weight z-score between 0-1 year.

In conclusion we have shown that TL is associated with neonatal abdominal circumference at birth and duration of breast-feeding. This suggests that reduced telomere length may be an early biomarker of the known association between early life nutrition and adult disease.

F-128

Uterine and Placental Adaptations and the Maintenance of Oxygen Consumption during Uterine Space Restriction. Ronald R Magness, Jason L Austin, Terrance M Phernetton, Katie M Meyer, Mary Y Sun, Jill M Koch, Jayanth Ramadoss, Pamela J Kling. *Ob/Gyn and Pediatrics, Univ of Wisconsin-Madison.*

Women with uterine anomalies and multi-fetal gestation have restricted uterine space and exhibit intrauterine growth restriction (IUGR). It is unclear how adaptations of the uterus vs the placenta affect local maintenance of oxygen consumption for fetal growth. Methods: We surgically created a didelphic/unicornuate ovine uterus (n=19) prior to conception (>60d) severing all inter-cornual connections followed by unilateral horn ligation/resection leaving the gravid horn locally exposed to fetoplacental factors and the nongravid horn exposed only to systemic factors. We obtained unilateral uterine blood flow (UBF) and uterine arterial & venous blood oxygen content on Gestational Day (GD) 120 and 130 (33 & 36 weeks' human equivalent) comparing data to Control Pregnant (n=17) and Nonpregnant (n=16) sheep. Results: Unilateral uterine surgery decreased maternal caruncle sites and # placentomes/horn by 35-50% and gestational UBF rises were not observed in nongravid horns, which were similar to Nonpregnants. Compared to Pregnant Controls, despite preconception reduction in uterine mass by unilateral surgery, uterine and placental growth responded by increasing (P<0.01) uterine myo-endometrial (29%) and placentomal (50%) weights, while maintaining UBF, uterine oxygen delivery, and oxygen consumption. Increased placentome weight in the unilateral group was solely due to an increase in fetal cotyledon (67%), not maternal caruncle (7%) weights. When >1 fetus was present in a horn, this was subcategorized as uterine space restricted (USR), and compared to non space restricted (NSR) exhibited 31% reduction of total placentome weight/fetus, but equal UBF and oxygen delivery even when expressed per metabolic tissue mass [uterine+placental+fetal]. By GD 130, USR fetuses exhibited growth arrest, smaller body mass, crown-rump, abdominal girth, fetal organs (heart, kidney, liver, etc.), but IUGR was asymmetric with brain sparing. Conclusions: We observed that only local, but not systemic, feto-placental factors are responsible for elevated UBF and fetal oxygenation. Although maternal caruncles were unchanged, the increased cotyledon size and compensatory uterine growth relative to total unilateral oxygen consumption reflect robust survival mechanisms for maintaining fetal growth even with reduced placental efficiency. NIH HL49210, HD38843, HL87144.

F-129

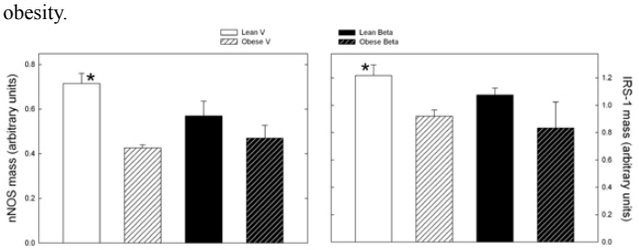
Effects of Antenatal Glucocorticoids and Diet-Induced Obesity on Skeletal Muscle Insulin Signaling Pathways. Angela G Massmann, Jeong-Heon Lee, Lourdes Flores, Jie Zhang, Jorge P Figueroa. *Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, USA.*

Antenatal exposure to glucocorticoids (GC) is associated with hypertension and alterations in glucose handling in adult life. Several mechanism have been suggested as explanation for the metabolic abnormalities. Skeletal muscle glucose utilization is an important component of insulin sensitivity and Nitric Oxide (NO) production by nNOS in skeletal muscle regulates glucose utilization. The aims of the present study were 1) to characterize the expression levels of nNOS and downstream insulin signaling molecules and 2) to determine if obesity exaggerates the effects of antenatal GC.

METHODS: Pregnant sheep were treated with two IM doses of betamethasone (Beta, 0.17 mg/kg) or vehicle (V 24-h apart at 80 days gestation and allowed to deliver at term. At 9 mo of age, sheep were randomly allocated to be fed at

either 100% of recommended nutritional allowance (Lean V/Lean Beta) or ad libitum (Obese V/Obese Beta) for three months. Skeletal muscle tissue was obtained at necropsy. Total nNOS, Akt and IRS-1 abundance was evaluated in slow twitch (supraspinatus; SS) skeletal muscle by western blot. Data Mean±SEM were analyzed by ANOVA and/or two sample t test.

RESULTS: Ad lib fed sheep gain > 40% of the original weight. As shown in the figure nNOS and total IRS-1 protein abundance was significantly different among the four groups by ANOVA. IRS-1 and nNOS protein abundance was highest in control (Lean V) and significantly decreased by GC exposure and obesity.



CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects in skeletal muscle capacity to regulate glucose metabolism. Beta sheep exhibit alterations in key molecules associated with glucose utilization finding which is consistent with decrease glucose tolerance, hyperinsulinemia and insulin resistance. These abnormalities are exaggerated by diet-induced obesity. Funding HL 68728 HD 047584

F-130

Upregulation of the Chemokine SDF-1 and Its Receptor CXCR4 in Frontal Cortex (FC) of Hypometabolic Fetuses (MNR) Enables Continued FC Development. Cun Li,[1] Lynn Xie,[1] Laura A Cox,[2] Peter W Nathanielsz,[1] Thomas J McDonald.[1] *[1]Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA; [2]Genetics, Texas Biomedical Research Institute, San Antonio, TX, USA.*

Introduction: In extreme conditions, animals depress metabolic rate for long term survival. In the present study, FC gene arrays showed MNR group baboon fetuses to be hypometabolic, i.e., 30 metabolic pathways significantly decreased ($Z > 1.99$), but also indicated that chemokines, small proteins with critical roles in neuronal migration, proliferation and axon pathfinding, and their receptors were significantly up-regulated ($Z = 2.9$). We hypothesized that peptide expression of the chemokine SDF-1 and its receptor CXCR4, would be increased in FC of MNR group fetuses in late gestation (G) to facilitate continued development in hypometabolism.

Methods: Pregnant baboons were fed *ad lib* (CTR) or 70% CTR global diet (MNR) from 0.16 - 0.9 G (term ~ 184 d) with tissue collection under general anesthesia. FC SDF-1 and CXCR4 expression determined by semi-quantitative immunohistochemistry (IHC), quantified by image analysis (Image J, NIH) for Fraction (% area immunostained) and Density (arbitrary units) and mRNA of flash frozen FC by Illumina arrays. Statistical analysis: Student's t-test, data as mean ± SEM, CTR data first, α level = 0.05.

Results: Fetal frontal cortex SDF-1 Fraction was significantly increased ($p < 0.01$), but Density was not ($p > 0.05$) nor was CXCR4 expression ($p > 0.05$) for Fraction or Density compared to CTR (Fig. 1).

Conclusions: The increase in SDF-1 and maintenance of CXCR4 expression in spite of MNR for 135 days, which produced global protein mRNA down-regulation, attests to the crucial roles of SDF-1 and CXCR4 in continued development of fetal brain.

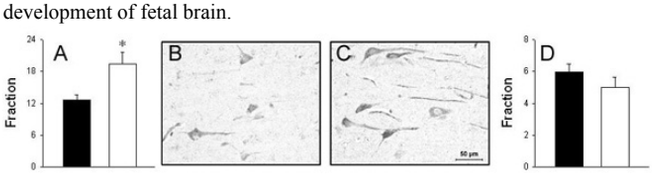


Figure 1. Summary (A) and photomicrographs (B & C) of SDF-1 and summary of CXCR4 (D) peptide expressed as Fraction determined by IHC and counting program in frontal cortex of fetuses from mothers *ad lib* fed (closed bars) or nutrient restricted (open bars; fed 70% CTR diet) from 30 – 165 days of gestation. Data expressed as mean ± SEM, * $p < 0.05$.

F-131

Effects of Antenatal Synthetic Glucocorticoid Treatment on Juvenile Behavior. Vasilis Moisiadis,[1] Alisa Kostaki,[1] Stephen G Matthews.[1,2,3] *[1]Physiology, University of Toronto, Toronto, ON, Canada; [2]Obstetrics and Gynaecology, University of Toronto, Toronto, ON, Canada; [3]Medicine, University of Toronto, Toronto, ON, Canada.*

Background: Approximately 10% of pregnant women are at risk of preterm delivery and receive synthetic glucocorticoids (sGCs) to reduce the risk of infant respiratory distress syndrome. Prenatal exposure to sGCs has been associated with modification of hypothalamic-pituitary-adrenal (HPA) function in first (F1) and second (F2) generation offspring in animals, and in humans has been linked to behavioral disturbance in young children. We hypothesized that prenatal sGC treatment leads to increased activity and reduced attention in juvenile F1 offspring.

Methods: Pregnant guinea pigs were subcutaneously treated with betamethasone (BETA; 1 mg/kg; n=16) or saline (Ctrl; n=15) on gestational days 40/41, 50/51, 60/61. Animals were allowed to deliver naturally (term ~ 69 days). Juvenile offspring were tested in an open field (30 min) to assess locomotor activity on postnatal days (PND) 19 and 24, and for prepulse inhibition (PPI; PND 23) as a measure of sensory motor gating (an index of attention).

Results: Prenatal exposure to sGC resulted in significantly increased activity in males and females on PND 24, compared to control offspring ($p<0.05$). Despite an increase in total activity, locomotor activity decreased in a linear manner over the test period at both ages ($p<0.01$) in sGC-exposed females. Activity did not decrease over time in control animals. In sGC-treated male offspring, activity remained elevated and did not decrease with time in the open-field. Prenatal sGC treatment increased PPI in juvenile male offspring ($p<0.05$), indicating increased attention levels in this group. There was no effect of sGC treatment on attention in female offspring.

Conclusions: Juvenile F1 offspring exhibit increased locomotor activity in an open field as a result of prenatal sGC treatment, and males display increased prepulse inhibition (increased attention). These differences in both locomotor activity and attention suggest altered signaling in dopamine-related brain regions. It is evident that prenatal treatment with sGC causes significant behavioral changes in juvenile offspring, and that these effects are sex- and age-specific. We are currently investigating the molecular mechanisms that underlie these behavioral changes in this well-characterized model.

Funded by: Canadian Institutes for Health Research.

F-132

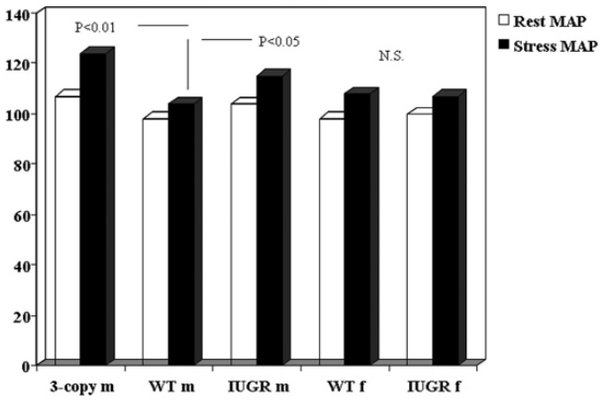
Fetal Programming of White Coat Hypertension in Growth Restricted Male Mice. Jessica Hebert, Ross Anderson, Elizabeth DuPriest, Terry Morgan. *Pathology and Obstetrics & Gynecology, Oregon Health & Science University.*

Background: Accumulating evidence suggests uteroplacental insufficiency and fetal growth restriction (IUGR) may cause reduced nephrogenesis and an increased risk of adult onset hypertension. This effect appears to be more pronounced in males than females. We study a transgenic mouse model designed to simulate a common human angiotensinogen (AGT) promoter variant associated with uteroplacental insufficiency and IUGR. Our objective was to test whether the wild-type progeny from transgenic dams have gender-based differences in nephrogenesis and develop adult onset hypertension.

Methods: Transgenic B6.129P2-Tg (Agtdup) mice (Jackson labs) were backcrossed for five generations into a wild-type C57BL/6 background, yielding female mice with 3-copies of the murine AGT gene. The progeny from 3-copy dams with wild-type (WT) AGT genotype (n=10 males and 12 females from five litters) were compared to transgenic 3-copy male positive controls (n=7) and gender-matched progeny from wild-type negative controls (n=22). Blood pressure was measured by radio telemetry in adult progeny (12 weeks old) at rest (2 hour mean arterial pressure [MAP]) and under mild stress for 30 minutes (loud hard-rock music, so-called "music torture"). Kidneys were harvested, weighed, and the number of glomeruli per kidney were determined by stereometry.

Results: Newborn pups from transgenic mothers were smaller ($p<0.05$), but caught up to controls by six weeks of age. Telemetry showed that IUGR males, but not IUGR females, had significantly elevated MAP when stressed, but not at rest compared with negative controls (WTm and WTf) (Figure). 3-copy male positive controls showed a similar increase in MAP, especially when stressed. Preliminary stereometric analysis suggested mean glomeruli/kidney counts may be significantly less in growth restricted males ($p<0.05$).

Friday



Conclusions: We observed adult onset increases in MAP in the IUGR male progeny from our transgenic mouse model, but only when these animals were stressed. The etiology may be related to differences in nephrogenesis, but we hypothesize there may also be differences in systemic sympathetic tone.

F-133

Promoter DNA Hypomethylation in the Fetal Kidneys of Intrauterine Growth Restricted Mice. Jessica Hebert, Emily King, Martha Choate, Elizabeth DuPriest, Terry Morgan. *Pathology, Oregon Health & Science University.*

Background: We have recently shown significant differences in fetal kidney renin-angiotensin system (RAS) expression in our mouse model of intrauterine growth restriction (IUGR). Similar to rat dams fed low protein diets whose progeny down-regulate renal renin expression, male progeny in our IUGR model also showed decreased renin and angiotensinogen (AGT) expression. Conversely, female IUGR progeny showed significantly increased AGT transcription. This may be significant because IUGR males have fewer nephrons and develop adult onset hypertension, while females do not. We hypothesize differences in renal RAS expression may be related to promoter methylation. Our objective was to characterize DNA methylation in fetal kidneys from our IUGR mice and controls.

Methods: Transgenic B6.129P2-Tg (Agtdup) mice (Jackson labs) were backcrossed for at least five generations into a wild-type C57BL/6 background, yielding female mice with 3-copies of the murine AGT gene. We harvested pregnant females near term (day 17) and collected fetal kidneys from five litters of each maternal genotype [3-copy; WT]. Fetal sex and genotype were determined by PCR. Only fetuses with 2-copies of the AGT gene were included for analysis. Global promoter methylation status was determined using methylated DNA immunoprecipitation coupled with microarray analysis (MeDIP-chip, Nimblegen). According to published criteria, promoters with DNA methylation levels lower than 1.3-fold of the genome-wide median methylation level were considered hypomethylated. Control (IgF2 DMR1) and RAS candidate genes were further analyzed by bisulfite pyrosequencing.

Results: Global promoter methylation was significantly less in IUGR female progeny compared to their IUGR male siblings and controls ($p<0.0001$). As expected, renal IgF2, H19, and XIST were hypermethylated in all fetal kidneys. There were no promoter methylation differences in renin, ACE, or the angiotensin II type-1 receptor between groups. However, the type 2 receptor (AT2) and ACE2 promoters were hypomethylated in IUGR progeny compared with controls. The AGT promoter was hypomethylated only in IUGR females.

Conclusions: Similar to recently published placenta data from human and mouse studies, we observed significantly less promoter methylation in the kidneys of our IUGR mouse model. There were also significant gender effects. IUGR female progeny tended to have less promoter methylation than their siblings and controls.

F-134

Aging of Isolated Pancreatic Islet Glucose (G) Stimulated Insulin (Ins) Secretory (GSIS) Response in Female Offspring (OFF) of Mothers Fed Low Protein (LP) Diet in Pregnancy (P) and/or Lactation (L). Sumiko Morimoto,[1] Tonantzin C Sosa,[1] Lizbeth Calzada,[1] Luis Reyes,[1] Eulises Diaz,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] *[1]Reproductive Biology, INNSZ, Mexico City, Mexico; [2]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

INTRODUCTION: Poor maternal nutrition impairs fetal and neonatal pancreatic islet growth, predisposing to failure of INS secretion. Maternal LP diet in P and/or lactation impairs male OFF isolated islet in vitro GSIS and accelerates islet aging (Br J Nutr. 2011: 1).

HYPOTHESIS: LP diet in P and/or L impairs isolated islet GSIS in female OFF leading to premature islet aging.

METHODS: We studied female OFF of rats fed control (C) (20% casein) or LP protein (R) 10% casein) isocaloric diets in pregnancy (first letter) and/or lactation (second letter) of four groups CC, RR, CR or RC. At postnatal day (PND) 36, 110 and 450, OFF were euthanized by decapitation and blood taken to measure serum G, INS and INS resistance index (IRI) and islets isolated (collagenase). In vitro GSIS was measured in 10 islets/well, in 5mM and 11mM G. After 1 h, medium collected and INS measured (RIA).

RESULTS: G and INS were normal in all groups and ages except PND 450, when INS and IRI in R groups were 32 to 84% higher than CC indicating INS resistance (data not shown). GSIS was blunted in R groups at 110 and 450 PND (Fig 1).

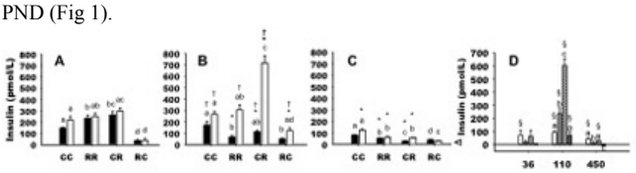


Fig 1. Female OFF GSIS from isolated islets (pmol/L/10 islets/60 min). Solid 5mM G and open 11 mM G. For groups see methods. Data M±SEM at A) PND 36(n=5), B) PND 110 9n=5), C, PND 450 9n=5) and D) Delta INS secretion between 5 and 11 mM G; In D - First bar CC, second RR, third CR and 4th RC. $p<0.05$ bars not sharing at least one letter at same age and G, *vs PND 36 † vs OBD 450 at the same G, §5 vs 11 mM in each group and age.

CONCLUSIONS: LP diet in P and/or L leads to reduction and premature aging of GSIS in female OFF. These findings are similar to those reported in male OFF but at all ages GSIS was better in females providing a partial explanation for the greater effects of programming on male OFF metabolism.

F-135

Infantile Growth after Undernourishment *In Utero* Positively Correlates with Fat Deposit in the Liver of Adult Mice, as a Risk of Non-Alcoholic Fatty Liver Disease (NAFLD). Keiko Muramatsu, Hiroaki Itoh, Yukiko Kohmura, Kaori Yamazaki, Kotomi Nagahashi, Yuki Nakamura, Naoki Tamura, Toshiyuki Uchida, Kazunao Suzuki, Naohiro Kanayama. *Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka-ken, Japan.*

Objective; The infantile rapid growth after undernourishment in utero was reported to be a risk factor of adult metabolic syndrome. In some cases of metabolic syndrome, fatty liver proceeds to Non-alcoholic Fatty Liver Disease (NAFLD) or Non-alcoholic Steatohepatitis (NASH), being at high risk of hepatic dysfunction as well as hepatoma.

In this study, we hypothesized that rapid growth during infantile period after undernourishment in utero is causatively associated with the increase of fat deposit in the liver under western life style. To prove the hypothesis, we developed a mouse animal model of undernourishment in utero and compared between the growth at weaning and weight of fatty liver and total cholesterol content in the liver.

Methods; Maternal caloric restriction (30% reduction) was apply to C57/BL6 pregnant mice. At 3 wks, body weight distribution of the offspring was assessed by the formula of [(body weight) – (mean body weight)] / standard deviation (SD) of body weight = SD ratio at weaning. After a HFD (60% fat) from 9 to 17 wks, weight of the liver, total cholesterol content in the liver, and oil red staining of the hepatic tissue were assessed.

Result; The mean birth weight of under nourished offspring was significantly lower than that of normally nourished offspring (85%, $p<0.001$). At 17 wks after HFD, weight of the liver ($r=0.87$, $p<0.01$) as well as total cholesterol content in the liver ($r=0.87$, $p<0.01$) was positively correlated with SD ratio at weaning (3 wks) in under nourished, but not normally nourished, offspring. In

under nourished offspring, hepatic pathology showed that hepatic cell swelling, i.e. hepatocelluer ballooning, concomitant with numerous lipid droplet, being compatible with NAFLD.

Conclusion; These findings indicate that the infantile rapid growth after undernourishment in utero accelerates deteriorating fatty liver.

F-136

Metabolic Aging of Offspring (OFF) Is Dependent on Maternal Age at Conception. Luis Reyes,[1] Rosalinda Charco,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] [1]*Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico;* [2]*OB/GYN, Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA.*

Background: Rat studies on developmental programming of metabolic disease in OFF have mostly been conducted in pregnancies with conception at maternal post-natal day (PND) 60 to 90 when mothers are still growing and compete with their fetal and neonatal young for nutrients.

Hypothesis: Programming of OFF metabolic outcomes differs by maternal age at conception.

Methods: Female rats were bred at PND 70, 90 or 150. All OFF were weighed weekly and for six male OFF (different litters) IVGTT was conducted at PND 600 in OFF fasted overnight. Glucose and insulin; area under the curve (AUC) were calculated. Animals were euthanized at PND 850. Fat depots were weighed. Adiposity index (AI): fat tissue weight (thoracic and visceral)/weight body * 100). Body weight (BW), food intake (FI), fat cell size, serum leptin, triglycerides (TG), cholesterol, glucose (GL), insulin and HOMA measured.

Results: Birth weight and body weight at PND 90 of OFF of PND 70 mothers were less than the other two groups. At the oldest ages studied (PND 600 and 850) OFF of PND 150 mothers had the lowest BW, and adiposity index (Fig 1) with no differences in FI. Fat content (70: 53±3a, 90: 62±1a, 150: 27±4b g) at PND 150 mothers eventually became the lowest while the other two groups were significantly higher. At 850 PND fat cell size (70: 17±1a, 90: 11±1b, 150: 12±0.9b x10^3μM) was bigger in OFF from 70 PND mothers. Leptin, TG, cholesterol, GL, and HOMA were not different at this advanced age.

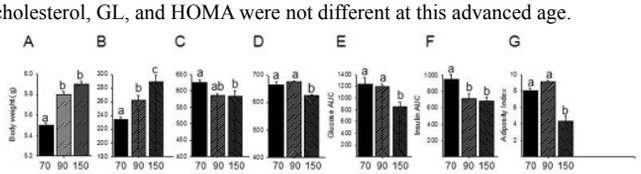


Figure 1. Panels A, B, C and D OFF A) BW - birth and PND (B) 90 (C) 600 (D) 850 (E). IVGTT: glucose AUC (F), insulin AUC (G) AI.

Conclusions: The role of maternal age in developmental programming has not been systematically studied. Our data clearly show that male OFF of the youngest mothers have the worst metabolic outcome in old age supporting our hypothesis that at PND 70 the intrauterine environment is less prepared for pregnancy than PND 150.

F-137

Increased Oxidative Stress Occurs Globally in Mother and Fetus in Response to Low Protein Diets in Pregnancy and Likely Constitutes a Fundamental Mechanism in the Altered Trajectory of Development: Effects of Resveratrol. Claudia Vega-Garcia,[1] Luis Reyes,[1] Omar Saldana,[1] Peter W Nathanielsz,[2] Fernando Larrea,[1] German Chamorro-Cevallos,[3] Elena Zambrano.[1] [1]*Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico;* [2]*OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA;* [3]*ENCB, Instituto Politécnico Nacional, Mexico City, Mexico.*

INTRODUCTION: Poor maternal diet in pregnancy alters the trajectory of development of multiple fetal organs predisposing offspring to a variety of chronic diseases in paot-natal life. One potential underlying mechanism is an increase in oxidative stress (OS) in the challenged fetal tissues.

HYPOTHESIS: OS represents a generalized mechanism resulting in altered fetal organ development.

METHODS: We studied female pregnant rats fed control (C – 20% protein) or low protein isocaloric (LP – 10%) diets in pregnancy. Resveratrol (RES) was administrated by gavage to pregnant mothers from day 0 to 19 (20 mg/Kg body weight/day). At 19 d of gestation (dG) rats were euthanized and maternal serum, placenta and fetal male brain and liver collected to quantify OS by malondialdehyde (MAD) by spectrometry. Statistics – two-way ANOVA, significance set at $p < 0.05$.

RESULTS: Maternal MAD increased in pregnancy compared with pre-pregnancy (Fig 1A) and further increased in LP an effect reversed by RES. LP increased placental, fetal brain and liver MDA also reversed by RES.

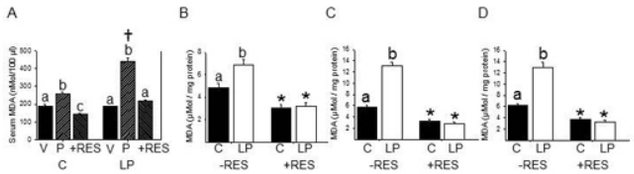


Fig 1. A) Maternal serum (B), placenta (C), fetal brain and (D) liver MAD. V: virgin, P: pregnancy, LP: Low protein, RES: resveratrol. M ± SEM, n=5, $p<0.05$ for groups not sharing at least one letter, * vs same group without RES . † vs pregnancy C.

CONCLUSION: Increased OS occurs globally in mother and fetus in response to LP diets in pregnancy and thus likely constitutes a common and fundamental mechanism in the altered trajectory of development. Maternal administration of RES reverses the negative effects.

F-138

Dietary Restriction in the Periconceptional Period in Both Normal Weight and Obese Ewes Results in Decreased Insulin Signalling in Skeletal Muscle of the Offspring. LM Nicholas,[1] JL Morrison,[1] L Rattanatray,[1] SE Ozanne,[2] D Kleeman,[3] S Walker,[3] S McLaughlin,[1] S Zhang,[1] IC McMillen.[1] [1]*Sansom Institute for Health Research, University of South Australia;* [2]*Institute of Metabolic Science, University of Cambridge, United Kingdom;* [3]*Turretfield Research Centre, SARDI, Australia.*

Maternal obesity is associated with reduced fertility, pregnancy complications and longer term consequences for her offspring including risk of obesity and insulin resistance (IR). Dietary restriction (DR) regimes have, therefore been proposed for obese women seeking to become pregnant to limit these effects. The impact of maternal obesity and of DR in the prepregnancy period in either normal weight or obese women on insulin signalling in metabolic tissues such as muscle in the offspring is unknown. We hypothesised that maternal obesity in the periconceptional (PC) period would result in decreased abundance of insulin signalling molecules in skeletal muscle of offspring and that DR in obese ewes would abolish these effects.

Donor ewes were allocated to 1 of 4 groups and fed the following diets in the PC period: 100% metabolisable energy requirements (MER) for ≥ 20wks (CC group); 100% MER for ≥ 16wks and then 70% MER for 4wks (CR group); ~180% MER for ≥ 20wks (HH group); ~180% MER for ≥ 16wks and then 70% MER for 4wks (HR group). These regimes continued for 1wk postconception before single embryos were transferred into recipient ewes of normal weight. Post mortem was conducted at 16wks postnatal age and quadricep muscle samples collected for determination of the abundance of insulin signalling molecules using Western blot analysis.

Maternal obesity in the PC period resulted in decreased GLUT4 abundance in muscle of female lambs. Maternal DR, however, resulted in increased insulin receptor abundance in muscle of offspring in both CR and HR groups. The abundance of PKCζ, phospho-AS160 and GLUT4 was lower, however, in muscle of CR and HR lambs compared to CC lambs. Furthermore, abundance of p110β was also decreased in CR lambs.

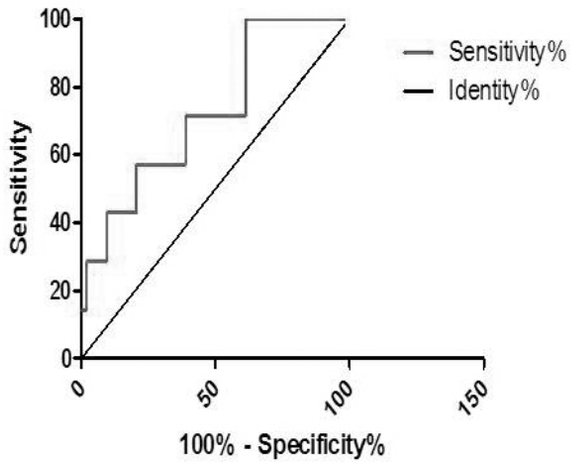
In conclusion, maternal DR in the PC period in normal weight and obese ewes results in decreased abundance of insulin signalling molecules in skeletal muscle of the offspring. These results suggest that maternal metabolic response to weight loss around conception, irrespective of her prepregnancy body weight has long lasting metabolic consequences for the offspring, which may contribute to the emergence of insulin resistance in later life.

F-139

Endocannabinoids and Pain: Endocannabinoid Levels in Women Presenting with Threatened Miscarriage Predict Outcome? Katerina N Bambang, David G Lambert, Justin C Konje. *Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Introduction: The endocannabinoid system composed of key ligands such as aracidonoylethanolamine (AEA) and oleylethanolamine (OEA) has been shown to be a key system in the regulation of blastocyst development, implantation and early pregnancy maintenance. A recent small pilot study suggested that AEA may be a biomarker for predicting miscarriage with elevated levels increasing the risk of early pregnancy loss in women presenting with painless vaginal

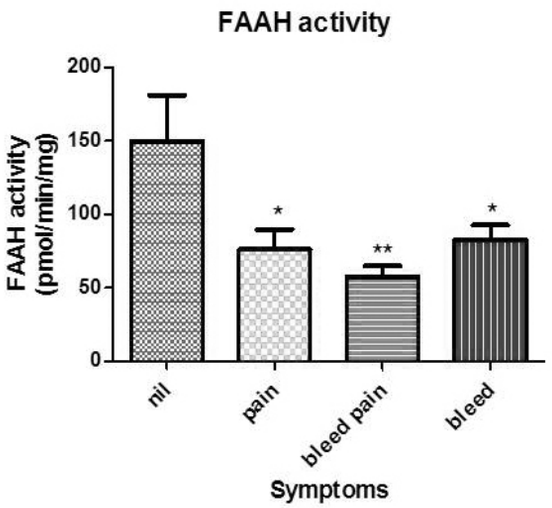
bleeding. The aim of this study was to assess the value of the endocannabinoids AEA and OEA in predicting pregnancy loss in women presenting to the EPAU with pain and a viable first trimester pregnancy.
Methods: Plasma levels of AEA and OEA were measured in 72 healthy pregnant women between 6-12 weeks gestation presenting to the EPAU with pain using UPLC-MS/MS and related to pregnancy outcome which was defined as miscarriage or live birth.
Results: Mean AEA levels in women with pain who miscarried (n=7) (0.67±0.17nM) were significantly higher than those who went on to have a live birth (n=65) (0.52±0.16nM) ($p<0.05$). An ROC curve of these results gives an area under the curve of 0.72. Using a cutoff level of 0.56 nM, a single plasma AEA measurement gives a sensitivity of 71% and a specificity of 61%.



Conclusion: Plasma AEA levels may be a biomarker for the prediction of miscarriage. This study is limited by the small number of participants but is currently being replicated in a larger cohort.

F-140

FAAH and NAPE-PLD as Gatekeepers of Plasma AEA Levels in Pregnant Women Presenting to the EPAU. Katerina N Bambang,[1] Monica Bari,[2] David G Lambert,[1] Mauro Maccarrone,[2] Justin C Konje.[1] [1]*Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom;* [2]*Dipartimento di Scienze Biomediche, Università degli Studi di Teramo, Teramo, Italy.*
Introduction: The endocannabinoid system composed of key ligands such as arachidonoylethanolamide (AEA) and oleylethanolamide (OEA) and the cannabinoid receptors (CB1, CB2), has been implicated as a critical component regulating early pregnancy. The synthetic enzyme NAPE-PLD and the degrading enzyme FAAH are thought to control AEA levels and it has previously been shown that their levels have a negative predictive value for miscarriage. Approximately 20-30% of women present to the EPAU with pregnancies symptomatic of threatened miscarriage.The aim of this study was to assess the levels of AEA in women with different symptoms and a viable pregnancy to determine if these correlate with synthetic and degrading enzyme activity.
Methods: Plasma levels of AEA as well as FAAH and NAPE-PLD activity were measured using UPLC-MS/MS in 44 healthy pregnant women between 6-12 weeks gestation presenting to the EPAU with symptoms of pain, bleeding, a combination of pain and bleeding or no symptoms.
Results: Mean AEA levels were highest (0.7717±0.31nM) in women with pain and lowest in those who were asymptomatic (0.5407±0.13nM). Conversely, FAAH activity was significantly lower in women presenting with both symptoms of bleeding and pain compared to asymptomatic women (149.9±82.65pmol/min/mg).



The synthesizing enzyme NAPE-PLD activity was highest (39.71±14.92pmol/min/mg) in asymptomatic women and similarly the degrading enzyme, FAAH was lowest (32.38±9.8pmol/min/mg) in women with pain and bleeding although there were no significant differences between the groups.
Mean AEA levels showed a large correlation with FAAH activity in those women with bleeding (r=0.55).
Conclusion: We report for the first time a significant variation in FAAH activity according to symptoms of threatened miscarriage in the first trimester. This is at present an unexplained finding. Interestingly, this is not the case with NAPE-PLD, further strengthening the suggestion that FAAH is the regulatory element of AEA metabolism.

F-141

Do Plasma Endocannabinoid Levels in Women Presenting with Threatened Miscarriage Predict Outcome? – An Update. Katerina N Bambang, David G Lambert, Justin C Konje. *Cancer Studies and Molecular Medicine, University of Leicester, Leicester, East Midlands, United Kingdom.*
Introduction: The endocannabinoid system which includes key ligands such as aracidonoylethanolamide (AEA) and oleylethanolamide (OEA) and the cannabinoid receptors (CB1, CB2), has been implicated as one of the critical components regulating early human pregnancy. A recent small pilot study suggested that the endocannabinoid, anandamide (AEA) may be a biomarker for predicting miscarriage. We have previously presented data on the use of AEA to predict miscarriage but were only able to report on women reaching 24 weeks gestation. We now report on women with a live birth or miscarriage. The aim of this study was to assess AEA as a predictor of miscarriage but also to determine the changes occurring in endocannabinoid levels in women presenting with a variety of symptoms to the EPAU.
Methods: Plasma levels of AEA and OEA were measured in 330 healthy pregnant women between 6-12 weeks gestation presenting to the EPAU with threatened miscarriage using UPLC-MS/MS and related to pregnancy outcome which was defined as miscarriage or live birth.
Results: Mean AEA levels in women with pain who miscarried (0.67±0.17nM) were significantly higher than those women with a live birth (0.52±0.17nM) ($p<0.05$). The mean plasma AEA level in women presenting with painless bleeding and live birth (0.58±0.21 nM) was not different from that in those with bleeding who miscarried (0.58±0.19nM) ($p>0.05$). Similar results were obtained with OEA.

Mean AEA and OEA levels in 251 women with threatened miscarriage

Symptoms	AEA (nM)		OEA (nM)	
	Miscarriage	Live Birth	Miscarriage	Live Birth
Bleeding	0.58±0.19	0.58±0.21	3.82±1.66	3.5±1.16
Bleeding/Pain	0.63±0.04	0.60±0.27	4.61±1.9	4.12±1.8
Pain	0.67±0.17	0.52±0.17	3.53±1.38	3.7±1.18
Asymptomatic	0.52±0.19	0.58±0.22	3.56±0.82	3.88±1.06

Conclusion: Plasma AEA levels in women presenting with pain to the EPAU have as in the past remained higher in those women who subsequently miscarry. This suggests the presence of different early pregnancy processes to those women presenting with bleeding or a combination of bleeding and pain.

F-142

Regulation of the Epithelial Membrane Bound Metalloproteinase ADAM17 in Human Endometrium and Fallopian Tube. Jeremy K Brown, Adam P Jowicz, Sarah E McDonald, Hilary OD Critchley, Andrew W Horne. *MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom.*

The "A Disintegrin And Metalloprotease" (ADAM) family of sheddases is thought to regulate a variety of cellular functions through proteolytic cleavage and shedding of cell surface proteins. ADAM17 has been proposed to participate in embryo implantation by regulating endometrial receptivity through the shedding of membrane bound mucin MUC1 by the endometrium. We hypothesised that ADAM17 would be up-regulated in human endometrium during the "window of implantation", but not in the Fallopian tube where implantation is pathological. Ethical approval for the study was obtained from the Lothian Research Ethics Committee (LREC 06/S1103/20), and informed written consent was obtained from all patients before sample collection. ADAM17 mRNA and protein expression were examined using Taqman quantitative RT-PCR, Western blot analysis and immunohistochemistry in carefully characterised endometrial (n=29) and Fallopian tube (n=12) biopsies obtained from non-pregnant women undergoing hysterectomy for benign gynaecological conditions. ADAM17 mRNA levels were also measured in immortalised endometrial epithelial cells (hTERT) stimulated with physiological concentrations of estrogen and progestogen. ADAM17 protein was primarily localised to the surface of the luminal epithelium in human endometrium and Fallopian tube. In the endometrium, ADAM17 expression was highest during the menstrual and proliferative phases of the cycle, dropping significantly at the onset of the secretory phase before steadily increasing again through the late secretory phase of the menstrual cycle. In contrast, there was no evidence for differential expression of ADAM17 across the menstrual cycle in the Fallopian tube. In vitro, ADAM17 mRNA was significantly upregulated by estradiol after 24 hours of treatment ($p<0.05$) but the progesterone analogue MPA did not significantly modify ADAM 17 expression. Our ex-vivo observations do not support a role for ADAM17 as a MUC1 sheddase during the "window of implantation" but suggest that further investigation of the precise function of ADAM17 in the female reproductive tract is warranted.

F-143

Understanding Tubal Ectopic Pregnancy: Anandamide Regulates ERK1/2 Phosphorylation through Cannabinoid Receptor-1 in Human Fallopian Tube but Does Not Directly Affect Tubal Smooth Muscle Contractility. Jeremy K Brown, Elizabeth M Oliver, Hilary OD Critchley, Andrew W Horne. *MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom.*

Background: The aetiology of tubal implantation is incomplete, but existing literature supports the hypothesis that it arises through a combination of impaired embryo-tubal transport and changes in the Fallopian tube (FT) epithelium that support pathological implantation. Endocannabinoids are multifunctional proteins thought to play a role in embryo transport through their effects on oviductal smooth muscle. Embryo retention in endocannabinoid receptor 1 (CB1) null mice and the association between attenuated CB1 expression in the human FT and ectopic pregnancy (EP) support this role.

Methods: Ethical approval for this study was obtained from the Lothian Research Ethics Committee (LREC 10/S1102/40), with informed written consent obtained from all patients. The response of human FT to the endogenous endocannabinoid ligand, anandamide (AEA), and the CB1 specific agonist arachidonyl-2-chloroethylamide (ACEA) was investigated in vitro. Explanted rings of human FT were exposed to 10e-5 to 10e-11M of AEA, ACEA or vehicle. Phosphorylation of mitogen activated protein kinases ERK1/2 was analysed by Western blot. Isometric muscle responses were amplified and digitized via an analogue/digital interface and analyzed using LabChart software (AD Instruments), using 10e-4 to 10e-9M of acetylcholine (ACh) as a positive control.

Results: Both AEA and ACEA induced rapid changes in ERK1/2 phosphorylation consistent with signaling through the CB1 receptor in vitro. However, neither agonist induced a detectable change in FT smooth muscle contractility in vitro, despite confirmation of the competence of the smooth muscle using ACh.

Conclusions: Our data suggest that AEA signals through CB1 in human FT but does not act in isolation to moderate FT smooth muscle contractility.

F-144

Serum Fibronectin as a Diagnostic Biomarker for Ectopic Pregnancy. Jeremy K Brown,[1] Katharina B Lauer,[1] Neil F Inglis,[2] Hilary OD Critchley,[1] Andrew W Horne.[1] *[1]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom; [2]Moredun Proteomics Facility, Moredun Research Institute, Edinburgh, United Kingdom.*

Background: Ectopic pregnancy (EP) has a major clinical and socioeconomic impact worldwide. Diagnosis of EP is often difficult and resource intense, with only half of cases positively diagnosed on first presentation. There is an unmet clinical and economic need for accurate biomarkers of EP.

Methods & Results: Ethical approval for this study was obtained from the Lothian Research Ethics Committee (LREC 04/S1103/20 and 09/S1103/39), with informed written consent obtained from all patients. A "shotgun" proteomics approach was used to interrogate pooled sera from women (18–45 years) undergoing surgical management of tubal EP (n=15), surgical termination of pregnancy (ultrasound-confirmed viable intrauterine, n=15) and surgical management of miscarriage (ultrasound-confirmed nonviable intrauterine, n=10). This approach identified a reduction in fibronectin levels in pooled serum from the ectopic group. Western blot analysis of individual sera confirmed that there was a significant reduction in serum fibronectin levels in patients undergoing surgical management of tubal EP ($P<0.01$: Mann Whitney). A competitive fibronectin ELISA was subsequently developed and validated for use on sera collected from a larger cohort of women (n=120: aged 18-50 years) at their first presentation in the first trimester of pregnancy with abdominal pain and/or bleeding and an inconclusive ultrasound scan. Although not statistically significant, the data show a clear trend towards reduced serum fibronectin levels in EP patients at the time of first presentation with symptoms in early pregnancy.

Conclusions: The data support inclusion of fibronectin in future multiplexed serum biomarker assays for the diagnosis of EP.

F-145

Hypoxia Is Not Required To Trigger Menstrual Breakdown of Human Endometrium. Pauline Coudyzer,[1] Benedicte Jordan,[2] Pascale Lemoine,[1] Christine Galant,[1] Maria-Luz Alvarez,[3] Aude Beliard,[3] Michelle Nisolle,[3] Jean-Michel Foidart,[3] Patrick Henriet,[1] Etienne Marbaix.[1] *[1]de Duve Institute, Université Catholique de Louvain, Bruxelles, Belgium; [2]Louvain Drug Research Institute, Université Catholique de Louvain, Bruxelles, Belgium; [3]Laboratory of Tumor and Development Biology, Université de Liège, Liège, Belgium.*

Menstruation is remarkable because tissue breakdown induced by estradiol and progesterone withdrawal is followed by scarless tissue regeneration. However, in vivo exploration of these physiological mechanisms is limited, because menstruation only occurs in few species. Vascular changes, in particular vasospasm of spiral arteries inducing ischemia and hypoxia, were implicated in triggering menstruation.

The aim of the project was to investigate whether hypoxia was needed to induce tissue breakdown and regeneration in an in vivo model of menstruation, using human endometrial tissue xenografted to ovariectomized immunodeficient mice. Three weeks after grafting, human endometrial tissue was vascularized and largely decidualized upon estradiol and progesterone treatment. Hormone withdrawal on day 21 after grafting induced menstrual breakdown within 3 to 4 days despite the absence of spiral arteries in the xenografts. Lithium phtalocyanate cristals implanted inside the endometrial fragments allowed to measure partial pressure of oxygen (pO2) within tissue by electron paramagnetic resonance (EPR). The pO2 level was measured on days 7, 14 and 21 after the graft and each day after hormone withdrawal (on day 21). Tissue was rather hypoxic 7 days after grafting (14.8 +/- 3.4 mm Hg) but significant reoxygenation occurred at the second week after grafting (day 14 : 21.5 +/- 5.5 mm Hg, day 21 : 21.6 +/- 5.7 mm Hg, n = 22-29 ; $P<0.00001$), when tissue was largely revascularized. Estradiol and progesterone withdrawal did not change tissue oxygenation during the first 3 days but unexpectedly increased pO2 levels 5 and 7 days after hormone pellets removal (30.7 +/- 4.1 vs 21.6 +/- 4.6 mm Hg at day 26; 29.8 +/- 9.5 vs 22.3 +/- 5.1 mm Hg at days 27-29, n = 3-17). These results were confirmed using ruthenium-based Oxylite measurements in 3 mice. We conclude that hypoxia is not needed to trigger menstrual tissue breakdown after estradiol and progesterone withdrawal in human endometrial xenografts.

F-146

Endometrial Repair and Regeneration: Studies in Mouse Models. Fiona L Cousins, Alison Murray, Hilary OD Critchley, Philippa TK Saunders. *MRC Centre For Reproductive Health, The University of Edinburgh, Edinburgh, United Kingdom.*

Background: The human endometrium is a dynamic sex steroid dependent organ subject to cyclical episodes of proliferation, shedding (menses) and repair. Studies of human endometrium have revealed that tissue restoration occurs in parallel with tissue breakdown. Menstruation is initiated following progesterone withdrawal. Mechanisms regulating repair/regeneration are unclear; recent studies in mice suggest they are estrogen (E) independent, a finding consistent with low levels of estrogens at menses. A role for endometrial progenitor (stem) cells in endometrial regeneration has been proposed with evidence from mouse models that estrogens increase their number. Wnts are proposed as mediators of stem cell proliferation in adult organs and are expressed in human endometrium. Wnt can substitute for E in stimulating proliferation of ERα positive cells in mammary tumour models. The present study uses a mouse model of menstruation to explore regulation of endometrial breakdown, repair and regeneration.

Methods: Ovariectomised adult mice received 3 daily injections of estradiol (E2, 100ng/100µl in peanut oil). A progesterone (P)-secreting pellet (100mg/ml) was inserted subcutaneously on day 6 with E2 injections (5ng/100µl) on days 6-8. Five hours after the last injection decidualization was induced in one uterine horn using oil whilst the contra-lateral horn acted as a control. Endometrial breakdown was initiated by removing the P pellet, 96h after oil injection. Uterine horns (control vs decidualized) were recovered for RNA extraction or fixed for immunohistochemistry.

Results: Endometrial breakdown was associated with blood in vaginal smears and detectable in the uterine lumen 7h after removal of the P pellet. Consistent with published data, uterine morphology was restored within 48h. Androgen receptor mRNA was present in uterine tissue regardless of decidualization status and was unaffected by P withdrawal. In contrast, expression of ERα mRNA was significantly increased in the decidualized horn 24h after P withdrawal ($n=4$, $P<0.01$). Preliminary data suggest tissue regeneration in this mouse model may be accompanied by increased expression of Wnt 5a.

Discussion: A mouse model of decidualization/P-withdrawal that recapitulates features of human endometrial menses associated with repair and restoration of tissue architecture highlights the potential for Wnt and androgen-dependent signalling pathways in uterine tissue regeneration.

F-147

Too Much of a Good Thing? The Effect of Precocious hCG on Endometrial Receptivity. Jemma Evans,[1] Luk JF Rombauts,[2,3] Lois A Salamonsen.[1,2] *[1]Uterine Biology, Prince Henrys Institute, Melbourne, Victoria, Australia; [2]Department of obstetrics and Gynecology, Monash University, Melbourne, Victoria, Australia; [3]Monash IVF, Melbourne, Victoria, Australia.*

Aim: Receptivity of the endometrium in assisted reproduction (ART) cycles is altered versus natural-cycles. A recent study examining frozen embryo transfers demonstrated lower ongoing pregnancy rates in women who received hCG to trigger ovulation vs. transfers following natural LH-surge (1). We therefore investigated if hormones administered during ART cycles, particularly hCG, cause the endometrium to become refractory to blastocyst signals at implantation.

Methods: Immunohistochemistry with semi-quantitative scoring identified localization and expression of the LH/CG receptor (R) in cycling endometrium (Proliferative, early-, mid- and late-secretory, n=10 per cycle stage) compared with endometrium from ART cycles at hCG+2 (GnRH antagonist not pregnant (np) n=10, GnRH agonist (np) n=16, GnRH agonist pregnant n=12). In vitro experiments aimed to mimic ART cycle exposure to hCG by administration of low doses of hCG (0.5 – 5IU hCG) for 5 days followed by a 'high dose' of hCG (20IU) mimicking blastocyst hCG secretion. LHCGR expression, activation of signaling (ERK 1/2 Western immunoblot), epithelial tight junction integrity (transepithelial resistance and tight junction immunocytochemistry) and cell adhesion (adhesion assay) were determined in the HES cell line in response to treatment with 0.5 - 5IU hCG for 3-5 days followed by 20IU hCG.

Results: Expression of LHCGR was decreased in glandular and luminal epithelium of the agonist np group vs early and mid secretory endometrium ($p<0.05$). In cells pre-exposed to hCG the LHCGR was down-regulated and re-localized to the nucleus. ERK 1/2 phosphorylation was reduced in response to 20IU hCG after pre-exposure to hCG. Epithelial tight junction integrity could not be 'relaxed' after pre-exposure to hCG, whereas treatment with 20IU alone mediated re-localization of ZO-1. Adhesion of endometrial epithelial cells to blastocyst like extracellular matrices (fibronectin, collagen I or IV) was reduced upon pre-exposure.

Conclusions: Exposure to hormonal regimens in ART cycles decreases LHCGR expression. Prior exposure specifically to hCG causes alterations in receptor expression and localization with the endometrial epithelium becoming refractory to blastocyst signals as indicated by physiologically-relevant assays.

1. Fatemi et al. Fertil Steril. 2010. 94(6):2054-8.

F-148

cAMP Stimulates Interleukin 11 mRNA Levels in Immortalized Human Endometrial Stromal Cells. Adam J Fechner, Donna M Cole, Andrea S Wojtczuk, Gerson Weiss, Laura T Goldsmith. *Obstetrics, Gynecology and Women's Health, UMDNJ-New Jersey Medical School, Newark, NJ, USA.*

Preparation of the maternal decidua for embryo implantation depends on the coordinated action of various local and circulating factors, several of which exert their effects by increasing intracellular levels of cAMP. The multifunctional cytokine, interleukin 11 (IL-11), is produced by the human endometrium and is critical for decidualization and implantation in mice. We have previously demonstrated that increased intracellular cAMP stimulates IL-11 protein expression in immortalized human endometrial cells using a PKA-independent pathway which involves MAP-kinase. However, data regarding the upstream regulation of this process are limited. The current study tested the hypothesis that increased intracellular cAMP stimulates IL-11 mRNA levels, leading to increased protein expression.

A well-characterized, telomerase-immortalized human endometrial stromal cell line was used. Cells were plated in phenol red-free media, serum starved for 24 hours and then incubated in the absence or presence of 0.5mM 8-br-cAMP for 4, 16, 20 and 24 hours. At each time point, conditioned media were collected from control and treated cells, and total cellular RNAs were isolated. Media IL-11 protein levels were determined using a human IL-11 specific ELISA. Quantitation of IL-11 and beta-actin mRNAs was performed by real time RT-PCR. Beta-actin was used as a reference gene as we have previously demonstrated that beta-actin mRNA levels are not regulated by increased intracellular cAMP in these cells. Comparison of relative mRNA levels between the treated and untreated cells was performed using the ΔΔCt method.

Both IL-11 mRNA and protein expression were upregulated by increased intracellular cAMP in a similar time-dependent manner, showing marked increases by 4 hours and peak levels at 20 hours, as shown in detail below (Table 1).

Table 1. Increased Intracellular cAMP Increases IL-11 mRNA and Protein

Fold Increases above Non-Treated Control				
	4 hours	16 hours	20 hours	24 hours
IL-11 mRNA	5.1	9.1	16.4	10.5
IL-11 Protein	1.8	7.0	12.1	3.5

These findings further elucidate the mechanisms which regulate production of a critical factor involved in endometrial function and embryo implantation and now allow for the hypothesis that cAMP-stimulated expression of IL-11 is transcriptionally regulated.

F-149

Endometrial Fluid Profiling during the Window of Implantation: A Noninvasive Approach to Studying Endometrial Receptivity. Rebecca Flyckt,[1] Tracey Bonfield,[2] Jeffrey Goldberg,[1] Tommaso Falcone,[1] Nina Desai.[1] *[1]Reproductive Endocrinology and Infertility, Cleveland Clinic, Cleveland, OH, USA; [2]Pediatric Pulmonology, Case Western Reserve University, Cleveland, OH, USA.*

Objective: Successful pregnancy requires both a good quality embryo and an endometrium that is receptive to implantation. Our objective was to determine whether quantitative analysis of cytokines and growth factors could be performed on a microvolume of fluid aspirated on the day of fresh or frozen blastocyst stage embryo transfer. A secondary objective was to assess whether specific markers were predictive of clinical pregnancy.

Design: Prospective cohort

Materials and Methods: This pilot analysis included 35 patients undergoing fresh or frozen blastocyst transfer between February 2011 and June 2011. Microvolumes of endometrial fluid were aspirated with a Wallace catheter under ultrasound guidance immediately before embryo transfer. Multiplex immunoassay was used to quantify 9 target cytokines and growth factors in the fluid. In vitro fertilization cycles resulting in pregnancy were compared to nonpregnant cycles, and fresh embryo cycles were compared to frozen cycles using the chi-square and Student's t-test as appropriate. P-values <0.05 were considered significant.

Results: 15 fresh cycles and 20 frozen cycles were analyzed. The groups did not significantly differ with respect to any descriptive or cycle characteristics. The overall clinical pregnancy rate for the study was 54% (19/35), consistent with our annual statistics. High endometrial fluid concentrations (>50pg/mL) were recorded for IL1a, IL6, IL8, MCP1, and VEGF, with the highest concentrations demonstrated by MCP1 (mean=695.5 ± 1117.4 pg/mL). Outliers beyond one standard deviation were removed. MCP1 had an inverse correlation with clinical pregnancy, the only cytokine that achieved statistical significance (218.9pg/mL vs 455.8pg/mL, p=0.045). Small concentrations (<10pg/mL) of GMCSF and TNFa were detected. IL1b and IL10 were not present.
Conclusions: Aspiration of endometrial fluid at the time of embryo transfer is a simple and safe approach for exploring endometrial receptivity. Noninvasive profiling of endometrial secretions is a novel technique which allows the study of heterogenous molecular mixtures in minute volumes without compromising pregnancy rates in IVF. Further, several target cytokines were present in high concentrations and may correlate with clinical pregnancy rates. Most notably, MCP1 appears to be negatively correlated with clinical pregnancy. Additional data collection is ongoing.

F-150

Annexin A2 Is Critical for Endometrial Adhesiveness and Embryo Attachment in Humans by Activation of RhoA through F-Actin Regulation.
Tamara Garrido-Gomez,[1] Francisco Dominguez,[1] Alicia Quinonero,[1] Carlos Estella,[1] Felipe Vilella,[1] Carlos Simon.[1,2] *[1]Fundacion IVI, Fundacion IVI, Instituto Universitario IVI, Universidad de Valencia, INCLIVA, Paterna, Valencia, Spain; [2]Stem Cell Bank, Prince Felipe Research Centre, Valencia, Spain.*
Introduction
Annexin A2 (ANXA2) has been found to be up-regulated in the human receptive endometrium by proteomic techniques (1). ANXA2 is linked to RhoA pathway regulating cell adhesion (2). In this work, we have demonstrated that ANXA2 regulates adhesiveness of the human endometrial epithelial cells (hEEC) to embryos through Rho GTPases regulation acting on F-actin rearrangement.
M&M
ANXA2 has been analysed in human endometrium (n=15) by IHQ and WB. We used an *in-vitro* model of adhesion to evaluate the attachment of mouse embryo and JEG-3 spheroids to HEC-1-A and hEEC. Inhibition of ANXA2 was performed by siRNA and Withaferin A. Activated/inactivation of RhoA was performed by transfection with constitutively active RhoA Q63L/dominant negative RhoA T19N or Toxin B treatment.
Results
ANXA2 is present in mid- and late-secretory endometrium, mainly localized to luminal epithelium. Inhibition of ANXA2 induces a significant decrease in adhesiveness compared to controls (60.3% in HEC-1-A and 85.48% in hEEC). ANXA2 was also implicated in endometrial epithelial cell migration by wound healing assay and trophoblast outgrowth. ANXA2 inhibition does not affect total RhoA levels, although significantly reduce active RhoA. Inhibition of RhoA also reduces embryo adhesiveness. The induction of constitutively active RhoA partially reverses the effects of ANXA2 inhibition on endometrial adhesiveness; however inactivation of RhoA does not alter ANXA2 levels. Deepening in the possible mechanism, we observed that these molecules co-localize in the apico-lateral plasma membrane of hEEC, and a high proportion of ANXA2 and RhoA are co-localized in the F-actin networks. Furthermore, the functional effects of ANXA2 inhibition and RhoA inactivation were associated with significant alterations in F-actin organization and their depolymerization.
Conclusion
ANXA2 may act upstream of the Rho pathway regulating F-actin remodeling, being a key factor of human endometrial adhesiveness. We suggest that ANXA2/RhoA pathway is a potential target to be explored for translational application to either improve embryo implantation or as a non-hormonal interceptive strategy.
1. Dominguez F et al. Human Reproduction 2009
2. Rescher U et al. J Cell Sciencie 2008

F-151

Endocannabinoids and Ectopic Pregnancy. Does Evidence of Chronic Inflammation in Fallopian Tubes Alter Plasma Endocannabinoid Levels?
Alpha K Gebeh, Jonathon Willets, Timothy Marczylo, Patricia Lam, Justin Konje. *Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*
Introduction
Endocannabinoids are a group of compounds that have been linked to the regulation of many biological processes in early pregnancy including embryo development and tubal transport. Ectopic pregnancy is one such condition that these compounds are thought to modulate. Previously, we have demonstrated that plasma levels of endocannabinoids [anandamide (AEA), oleoylethanolamide; (OEA), palmitoylethanolamide (PEA)] are elevated in ectopic pregnancy compared to normal pregnant controls. Here, we evaluated whether or not levels are significantly different between those with and without evidence of chronic inflammation; a recognized risk factor for ectopic pregnancy.
Methods
Plasma endocannabinoid levels were measured in women with ectopic pregnancy (n = 38) after solid-phase extraction and quantified by UHPLC-ESI-MS/MS, utilizing an isotope dilution method and selective ion monitoring. Samples were categorized into 2 groups i.e. those with salpingitis or those with normal fallopian tubes based on the histology report following salpingectomy.
Results
There was no significant difference in maternal age, body mass index (BMI) and gestation between the groups. Mean (±SEM) plasma endocannabinoid levels were not significantly different (p>0.05, Mann-Whitney U test) in those with chronic salpingitis (n=13) compared to those without (n = 35); AEA = 0.74 ± 0.06 versus 0.79 ± 0.06 nM, OEA = 5.60 ± 0.67 versus 4.99 ± 0.18 nM, and PEA = 14.93 ± 2.51 versus 12.82 ± 1.61 nM respectively.
Conclusion
The results suggest that the higher plasma levels previously observed in ectopic pregnancy compared to normal intrauterine pregnancy appears unrelated to chronic inflammation. In those women with normal fallopian tubes who develop ectopic pregnancy, dysfunction in the ECS may be an underlying cause. Further research is warranted into the role of the ECS in tubal transport as it may unravel new treatments and therapies.

F-152

Importance of Suitable Reference Gene Selection for qRT-PCR: Special Reference to Studies on Expression of the Endocannabinoid System in Human Endometrium.
Alpha K Gebeh,[1] Emma Marczylo,[2] Akwasi Amoako,[1] Kate Dudek,[2] Jonathon Willets,[1] Justin Konje.[1] *[1]Cancer Studies & Molecular Medicine, Reproductive Sciences Section, University of Leicester, Leicester, United Kingdom; [2]System Toxicology, MRC Toxicology Unit, Hodgkin Building, University of Leicester, Leicester.*
Introduction
Quantitative real time PCR (qRT-PCR) is commonly employed in gene expression studies including those evaluating the role of the endocannabinoid system (ECS) in ectopic pregnancy. Most of these studies use 18S, β-actin or GAPDH without validation. A systematic study of the suitability of reference genes for gene expression studies relating to the ECS in ectopic pregnancy is lacking. This study evaluated the suitability of 12 commonly used genes (18S, GAPDH, β-actin, ATP5B, YWHAZ, TOP1, UBC, CYC1, EIF4A2, SHDA, RPL13A & B2M) for normalization of qRT-PCR data and the effect of choice of reference gene on N-acyl-phosphatidylethanolamide phospholipase D (NAPEPLD) mRNA expression in human endometrium.
Methods
The mRNA expression stability of the panel of 12 genes were tested to identify the most suitable reference genes for normalization in 12 endometrial samples (ectopic pregnancy n=4; follicular n=4 and luteal n=4 phase non-pregnant women) using the geNorm reference gene selection kit (www.primerdesign.co.uk). qRT-PCR was performed using specific primers for each reference gene and the gene of interest (NAPEPLD) with SYBR green mastermix as the detection system. Relative expression values were exported to geNorm and NormFinder software for analysis.
Results
The genes consistently ranked in the top 6 by both software were UBC, ATP5B, SHDA & TOP1. The best pair genes were UBC & YWHAZ (geNorm) or UBC & ATP5B (NormFinder) (Table 1). NAPEPLD mRNA expression varied between the groups depending on which of the 12 genes was used as internal controls unlike those recommended by either software.

Conclusions

This study suggests that arbitrary selection of reference genes without validation should be discouraged and validation of reference gene stability should be undertaken prior to every study.

Ranking of reference genes in endometrium according to their stability value

Rank	Normfinder		geNorm	
1	CYC1	0.074	UBC	0.481
2	ATP5B	0.081	TOP1	0.504
3	UBC	0.088	SHDA	0.510
4	B2M	0.100	CYC1	0.514
5	TOP1	0.106	ATP5B	0.514
6	GAPDH	0.106	EIF4A2	0.517
7	SHDA	0.113	ACTB	0.533
8	YWHAZ	0.121	YWHAZ	0.542
9	ACTB	0.125	GAPDH	0.567
10	18S	0.144	18S	0.586
11	EIF4A2	0.148	RPL13A	0.672
12	RPL13A	0.148	B2M	0.774
Best pair	UBC & ATP5B		UBC & YWHAZ	

F-153

Fallopian Tube Endocannabinoid Levels and Expression of Their Metabolizing Enzymes in Ectopic Pregnancy. Alpha K Gebeh, Jonathon Willets, Anthony Taylor, Justin Konje. *Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Introduction

Understanding of ectopic implantation within the fallopian tube is limited though endocannabinoids (e.g. anandamide, AEA; oleoylethanolamide, OEA; palmitoylethanolamide, PEA) are thought to play a role. AEA activates cannabinoid receptors and together with its synthesizing [*N*-acylphosphadityl ethanolamine-specific phospholipase D; NAPEPLD] and degradative [fatty acid amide hydrolase; FAAH] enzymes form the endocannabinoid system (ECS). The ECS maintains a normal "anandamide tone" that permits embryo-tubal transport in rat models. Dysfunction of the ECS may underlie ectopic pregnancy; a condition associated with altered embryo-tubal transport. Here we quantified these compounds and evaluated the expression of FAAH & NAPEPLD in fallopian tubes.

Methods

Total RNA was reversed transcribed from 28 fallopian tubes (ectopic pregnancy n=8, follicular n=10 & luteal n=10 non-pregnant women). Standard qRT-PCR methods were employed using specific primers for FAAH & NAPEPLD. Immunohistochemistry was used to localize FAAH & NAPEPLD while western blotting methods were used to evaluate protein expression with FAAH / NAPEPLD transfected HEX cells as positive controls. Endocannabinoids were extracted from fallopian tubes using a solid-phase method and quantified by UHPLC-MS/MS.

Results

AEA (mean ± SEM) but not OEA and PEA levels were significantly higher ($p<0.05$ one-way ANOVA) in ectopic pregnancy than in luteal phase of controls (Table 1). There was no significant difference in levels across the different parts of tube. FAAH but not NAPEPLD mRNA was significantly lower ($p<0.05$ oneway ANOVA) in ectopic pregnancy compared to luteal phase controls. Immunohistochemical scores were consistent with mRNA data. FAAH & NAPEPLD was localized to the tubal epithelium.

Conclusions

This is the first human study to show dysregulation of AEA and its enzymes suggesting that the machinery maintaining the "endocannabinoid tone" required for normal embryo-tubal transport may be perturbed in women with ectopic pregnancy. The results of the effects of high anandamide levels on fallopian tube function (ciliary beat frequency and beat pattern) is currently being analyzed.

Endocannabinoid levels in fallopian tubes

	Follicular (n=6)	Luteal (n=9)	Ectopic (n=9)	P value
AEA	3.12 ± 0.48	2.74 ± 0.20	4.26 ± 0.36	0.03
OEA	16.12 ± 1.1	17.42 ± 3.2	27.80 ± 11.58	0.53
PEA	89.21 ± 16.75	116.3 ± 27.9	183.3 ± 79.32	0.80

F-154

Ectopic Pregnancy Is Associated with Downregulation of Type 1 Cannabinoid Receptor (CB1). Alpha K Gebeh, Jonathon Willets, Emma Marczylo, Justin Konje. *Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Introduction

Aberrations in the endocannabinoid system (ECS) are strongly linked to defects in embryo-tubal transport culminating in the retention of embryos in mice. The ECS comprise cannabinoid receptors (CB1, CB2), ligands (e.g. anandamide), synthesising (N-acylphosphadityl-ethanolamine-specific phospholipase D; NAPEPLD) and degradative (fatty acid amide hydrolase; FAAH) enzymes. In the only human study to date, CB1 mRNA has been shown to be reduced in ectopic pregnancy but no studies have evaluated the expression of CB2 receptors. Here, we evaluated the expression of CB1 & CB2 in ectopic pregnancy.

Methods

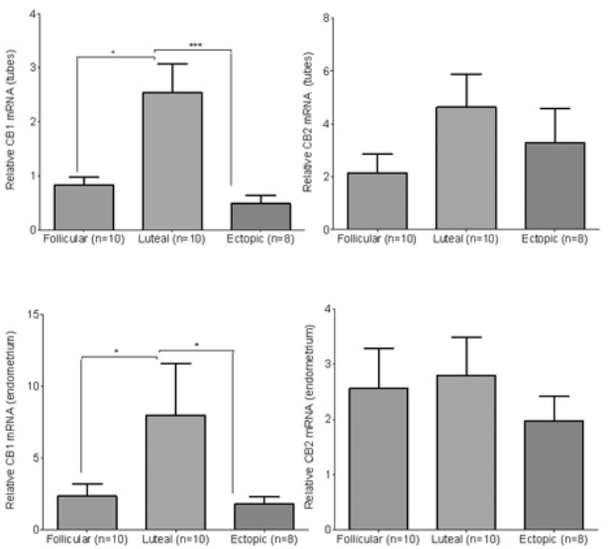
1g total RNA was reversed transcribed from fallopian tubes and endometrium from ectopic pregnancy (n=8), luteal (n=10) and follicular (n=10) phase non-pregnant women. RNA quality was assessed using an Agilent bioanalyzer and qRT-PCR performed using standard methods. Immunohistochemical methods were employed using polyclonal antibodies to localize CB1 and CB2. Antigen retrieval was performed for CB1 using proteinase K but was not required for CB2. CB1 and CB2 expression was quantified using an image analysis software.

Results

CB1 mRNA was significantly lower in ectopic pregnancy and the follicular phase compared to luteal phase in both the endometrium and fallopian tubes ($p<0.05$, one-way ANOVA) (Figure 1). There was no difference in CB2 mRNA expression between the groups ($p>0.05$ one-way ANOVA). CB1 and CB2 proteins were expressed in the cytoplasm but not the nuclei in tubal epithelium. CB1 and CB2 was expressed in both the stroma and glandular components of endometrial tissue. Immunohistochemical scores for CB1 and CB2 expression were consistent with mRNA data.

Conclusions

The results suggest that cannabinoid receptors may be involved in modulating ectopic pregnancy and confirm previous studies of a reduction in CB1 mRNA in ectopic pregnancy. CB2 on the other hand does not appear to be differentially regulated in ectopic pregnancy.



F-155

UBC, CYC1, EIF4A2 and GAPDH Are Suitable Housekeeping Genes for Quantitative Expression Analysis by Real Time PCR in Fallopian Tubes from Ectopic Pregnancy. Alpha Gebeh, Emma Marczylo, Jonathon Willets, Justin Konje. *Department of Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Introduction

Quantitative real time PCR (qRT-PCR) is commonly employed in gene expression studies in ectopic pregnancy as it offers the advantage of high sensitivity, specificity, ease of use and broad dynamic range. Although most studies employ 18S, β-actin or GAPDH as housekeeping genes, there is usually no evidence of validation of these genes prior to their use. This is particularly important because expression of housekeeping genes can be influenced by disease processes. This study tested 12 commonly used genes (18S, GAPDH, β-actin, ATP5B, YWHAZ, TOP1, UBC, CYC1, EIF4A2, SHDA, RPL13A & B2M) for their suitability as housekeeping genes.

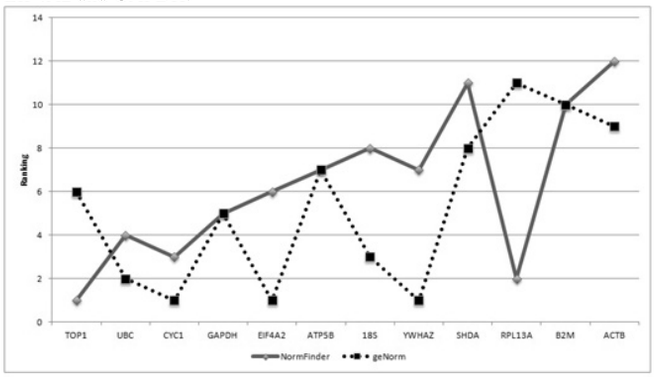
Methods

The mRNA expression stability of 12 commonly used housekeeping genes were tested to identify the most suitable genes for normalization in 12 fallopian tube samples (ectopic pregnancy n=4; follicular n=4 and luteal n=4 phase non-pregnant women) using the geNorm reference gene selection kit. The RNA was treated with DNase and the RNA quality tested with an Agilent bioanalyzer

prior to reverse transcription. qRT-PCR was performed using specific primers for each of the 12 genes. Relative expression values were exported to geNorm and NormFinder software for analysis.

Results

The ranking of the housekeeping genes were as follows (starting from the most stable to the least stable): TOP1 > RPL13A > CYC1 > UBC > GAPDH > EIF4A2 > ATP5B > 18S > B2M > YWHAZ > SHDA > ACTB (NormFinder) and EIF4A2 > CYC1 > UBC > 18S > B2M > GAPDH > TOP1 > ATP5B > ACTB > SHDA > YWHAZ > RPL13A (geNorm). The best pair of genes were UBC and TOP1 (NormFinder) and UBC, CYC1 and EIF4A2 (geNorm). The genes ranked consistently in the top 6 by either software were UBC, CYC1, EIF4A2 and GAPDH.



Conclusion

We recommend that arbitrary use of reference genes should be avoided and validation of reference genes undertaken prior to qRT-PCR studies. In our study, UBC, CYC1, GAPDH and EIF4A2 were consistently highly ranked by either software suggesting they may be suitable housekeeping genes for normalization.

F-156

Fibroblast Activation Markers in Ectopic and Eutopic Endometrium from Women with Endometriosis. Lulu Fu,[1,2] Michal Amir,[3] Peter AW Rogers,[2] Jane E Girling.[2] *[1]Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia; [2]Obstetrics and Gynaecology, The University of Melbourne, Melbourne, Victoria, Australia; [3]Monash Medical Centre, Melbourne, Victoria, Australia.*

Background: Fibroblasts have essential roles in deposition of extracellular matrix and regulation of inflammation and are therefore central to wound healing, scar formation and fibrosis, all processes that are central to the pathology of endometriosis. Our aim was to characterise the activation status of stromal fibroblasts in eutopic versus ectopic endometrium from women with endometriosis.

Methods: Endometriotic lesions and eutopic endometrial samples were collected from women undergoing laparoscopy for the treatment of endometriosis (n=4 proliferative, n=7 secretory). Sections containing endometrial glands were immunostained for CD10 to identify endometrial stromal cells. Laser capture microscopy was then used on serial sections to collect glandular epithelium and CD10-postive stroma from ectopic lesions and eutopic endometrium, as well as CD10-negative tissue from around ectopic lesions. After confirming the quality of extracted RNA, quantitative PCR was used to examine the expression of selected genes involved in fibroblast activation (TGFβ1, NFκB1, ACTA2, IFNγ, SMAD2, 3 and 4). mRNA levels were expressed relative to an RNA spike or 18S rRNA.

Results: Expression of TGFβ1 and SMAD4 mRNA was significantly higher in eutopic versus ectopic glandular epithelium and SMAD3 was significantly higher in secretary-phase eutopic glandular epithelium relative to all other groups. However, there was no significant difference in the expression of these genes in eutopic stroma, ectopic CD10-positive stroma or ectopic CD10-negative stroma. There was no significant variation in ACTA2, NFκB1, or IFNγ mRNA expression among any regions examined.

Conclusions: Variations in TGFβ1, SMAD3 and SMAD4 mRNA expression in eutopic relative to ectopic glandular epithelium are consistent with a specific function for the TGFβ pathway in eutopic endometrium that is not replicated in ectopic lesions. However, our mRNA data do not support a difference in fibroblast activation status between CD10-positive endometrial stromal fibroblasts in ectopic lesions versus eutopic endometrium or tissues adjacent to CD10-positive lesions. Further studies examining the protein expression of key fibroblast activation markers are underway.

F-157

Reducing Animal Suffering? A Novel 3D Ex-Vivo Model To Study Endometrial Epithelial Cell Biology. DK Hapangama, A Rak-Raszewska, M Simic, A Valentijn, P Murray. *Institute of Translational Medicine, University of Liverpool.*

Introduction: Characterization of human endometrial epithelial cells (hEEpCs) is fundamental to all research in Gynaecology. A hEEpC sub-population that is able to give rise to endometrial-like glandular architecture in vitro or in animal models remains to be identified. Recently, a protocol for studies on renal development has been established in which murine embryonic kidney cells (MEKCs) are dissociated and then re-aggregated to form organotypic renal structures. Since both the endometrium and kidneys are derived from the intermediate mesoderm, using this approach, we formed novel mouse-human ex-vivo chimeric culture system that allows the examination of the differentiation potential of singly dispersed hEEpCs in a more physiological 3D-environment.

Methods: Embryonic day 13.5 kidney rudiments were disaggregated to single cells, and re-aggregated in the presence of hEEpCs to form chimera. The disaggregated MEKCs were mixed at 1:10 ratio with the sorted primary hEEpCs and cultured on a 1.2 mm nucleopore membrane filter (n=4). Cells were labelled with species specific antibodies and endometrial gland-like organoids that developed were assessed by confocal microscopy for architectural organisation akin to human endometrial glands *in-vivo* after 10-14 days in culture. qPCR was performed to distinguish the respective genes expression, using human and mouse specific primers that were designed for regions of maximum difference.

Results: Singly dispersed primary EEpCs were able to give rise to gland-like structures in the kidney chimera system. A panel of human endometrial and renal specific genes (PR, MUC1, Synaptopodine, WT1, Pax2) were up-regulated in the hEEpCs during the development of the chimera compared to the monolayers. The chimeric growth also up-regulated endometrial specific PR & MUC1 in the MEKCs.

Conclusion: The development of endometrial glandular-like structures in the chimera suggests the presence of stem/progenitor cells in our primary hEEpCs. This model is more accessible and reproducible compared to the xenotransplant and animal (rodent or primate) endometrial re-generation models that have been used. Therefore, we propose that this model not only provides a means of analysing EEpSPC function and effects on exposure to drugs but it may obviate extensive in vivo experimentation in animals reducing animal suffering.

F-158

Unique Cellular Immune Environment of Endometrial Polyps. Tania El-Hamarneh, Alison J Hey-Cunningham, Marina Berbic, Ian S Fraser, Kirsten Black. *Obstetrics, Gynaecology and Neonatology, The University of Sydney, Sydney, NSW, Australia.*

Introduction

Endometrial polyps (EPs) are localized outgrowths of the surface endometrium, a common benign gynecological condition associated with symptoms such as abnormal uterine bleeding and infertility. The exact pathogenesis of EPs is unknown but a recent retrospective study has shown highly significantly increased density of activated, tryptase positive mast cells (MCs) compared with normal endometrium. It is not known whether increased MC density extends to the uninvolved endometrium, or whether other immune cell types are implicated.

Objective

To study the numbers and types of MCs and regulatory T cells (Tregs) in EPs, polyp-adjacent (adjacent) and -distant (distant) endometrium compared to normal endometrium.

Methods

Samples of EPs, adjacent, distant (n=23) and control endometrium (n=40) were prospectively collected from women of reproductive age. Tryptase, chymase and c-kit positive MCs and Foxp3 positive Tregs were quantified by immunohistochemistry.

Results

Densities of tryptase+, chymase+ and c-kit+ MCs were highly significantly increased in EP compared to adjacent (all $p<0.001$), distant (all $p\leq0.001$) and control endometrium (all $p<0.001$). Foxp3+ Treg density was increased in EPs compared to distant and control endometrium (both $p=0.002$). Chymase+ and c-kit+ MCs were increased in density in adjacent compared to control endometrium ($p=0.025$ and $p=0.003$, respectively). C-kit+ MCs were increased but Tregs decreased in distant compared to control endometrium ($p=0.019$ and $p=0.002$, respectively).There were no significant differences in MC or Treg density between adjacent and distant endometrium.

Friday

Conclusion
This study provides novel insights into localized disturbances in the cellular immune environment within EPs with significantly increased densities of MCs expressing a range of functional markers and Tregs. Tregs are likely to be recruited to EPs in an attempt to suppress the inflammatory processes due to the greatly increased presence of MCs. These immunological disturbances are almost certainly related to the abnormal bleeding and infertility that may occur in pre-menopausal women with EP.

F-159

Transcriptomics of Early Embryonic Invasion at Implantation Sites in a Murine Model. Juan M Moreno-Moya,[1] Anahi Franchi,[2] Sebastian Martinez-Escribano,[1] Jose A Martinez-Conejero,[1] Silvina Bocca,[2] Sergio Oehninger,[2] Jose A Horcajadas.[3] *[1]Fundación IVI-Instituto Universitario IVI-Universirty of Valencia, University of Valencia, Valencia, Spain; [2]The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, USA; [3]Araid at I+CS, Hospital Miguel Servet, Zaragoza, Spain.*
BACKGROUND
The embryonic invasion process requires two elements, one embryo that exerts the invasion and a maternal tissue that allows implantation and invasion. Disturbance in this dialogue is a potential source of serious pathologies such as pre-eclampsia and spontaneous abortion that can occur in early pregnancy.
OBJECTIVE
The aim of this study is to break through the transcriptomic of the early embryonic invasive process and the maternal control exerted by the surrounding decidual tissue.
DESIGN
Three pregnant female mice at 6.5 dpc were used to obtain tissue from invasive extra-embryonic tissue (ET) formed by the ectoplacental cone and two different decidual tissues, the surrounding decidua (SD) and the deciduas from the inter-implantation sites (ID).
MATERIALS AND METHODS
Gene expression was analyzed using gene expression microarray technology and RT-PCR was performed for microarray validation. DAVID and IPA softwares were used to analyze the Gene Ontology (GO) terms most represented in the differentially expressed genes.
RESULTS
Eight hundred seventeen genes were found to be differentially expressed between the ET versus SD and 360 genes between ID versus SD. Both comparisons shared 123 genes. Expression of 8 of the most differentially expressed genes was confirmed by RT-PCR. GO analyses showed that developmental and proliferation processes were over-represented in the differentially expressed genes in ET vs SD and immune cell trafficking in ID vs SD. A very interesting network of genes involved in replication, recombination and cell repair, cell death and cell growth was found in the comparison ET vs SD.
CONCLUSIONS
Our results provide closest information to humans about the transcriptome of the early embryonic invasion process at the implantation site and the control exerted by the surrounding decidual tissue. These results would be useful to find targets involved in different pathologies associated with implantation failure or early pregnancy loss.
SUPPORT
Ministerio de Ciencia e Innovación of the Spanish Goverment (SAF2008-04349).

F-160

Transforming Growth Factor Beta 1 (TGFβ1) and Progesterone Regulate Matrix Metalloproteinases (MMPs) in Human Endometrial Stromal Cells. Hiroko Itoh, Ruth A Word. *Obstetrics and Gynecology, University of TX Southwestern Medical Center, Dallas, TX, USA.*
Menstruation is preceded by progesterone withdrawal and endometrial matrix remodeling predominantly through induction of MMPs and recruitment of invading neutrophils. During the late luteal phase (a time in which progesterone levels decrease substantially), upregulation of proteases coincides with increased expression of several transforming growth factor β (TGFβ)-responsive genes. Here, we tested the hypothesis that TGF-β1 and progesterone regulate expression of MMPs and PR isoforms in human endometrial stromal cells.
Methods: Human endometrial stromal cells (HESC) were isolated and cultured from endometrial tissues in the secretory phase. Gene expression was analyzed using qPCR, and MMP2 and MMP9 activity was assessed using quantitative gelatin zymography. PR expression was analyzed using immunoblotting.
Results: Using endometrial tissues from women during various phases of the menstrual cycle, we found that MMP2 was upregulated in the late secretory phase and MMP-9 was upregulated just prior to menstruation. In contrast, MMP11 gene expression was suppressed dramatically during the secretory phase relative to high mRNA levels in the proliferative phase. To determine if TGFβ1 regulates MMP2, MMP9, or MMP11, endometrial stromal cells were treated with vehicle or TGFβ1 (0.1 – 5 ng/ml) for 48 h and mRNA levels were quantified using qPCR. TGFβ1 resulted in modest, but significant, increases *MMP2 and MMP9* (2 to 2.5-fold), increased pro- and active MMP-2 activity, and dramatic upregulation of *MMP11* gene expression (28-fold, $P < 0.01$). Progesterone inhibited TGFβ1-induced stimulation of *MMP2* and *MMP11* mRNA. Interestingly, TGFβ1 also decreased PR-A and PR-B in HESC with a more pronounced effect on PR-A. **Conclusions:** These data support the hypothesis that TGFβ1 has endogenous antiprogestational effects in HESC and that the opposing effects of progesterone and TGFβ1 are important in regulation of matrix integrity in human endometrium.

F-161

The Effect of Exogenous Administration of the Endocannabinoid Anandamide (AEA) on Implantation. Tulay Karasu,[1] Timothy H Marczylo,[1] Bruno M Fonseca,[2] Georgina Correia-da-Silva,[2] Natercia A Teixeira,[2] Justin C Konje.[1] *[1]Endocannabinoid Research Group, Reproductive Sciences, CSMM, University of Leicester, Leicester, United Kingdom; [2]Biochemistry, Faculty of Pharmacology, University of Porto, Porto, Portugal.*
Introduction: Endocannabinoids (EC) are unsaturated fatty acid derivates that play a pivotal role in reproduction via endocannabinoid receptor (CB1, CB2)-mediated cell signalling. AEA is important for fertilisation, implantation and early pregnancy maintenance. Low levels of AEA are present at implantation sites. Here we investigate the effect of exogenous AEA on implantation in rat.
Methods: A single injection of AEA (1mg/kg i.p.) or vehicle was given to pregnant Wistar rats on Day2, Day4 or Day6. The rats were sacrificed on Day14, implantation sites counted and the expression of the EC system in deciduas and placenta was determined by qRT-PCR.
Results: Rats treated with AEA on days 4 and 6 had a 14 and 33% decrease in the number of implantation sites, respectively compared to control animals. The left uterine horn, close to the site of injection, had less implantation sites than the right uterine horn.

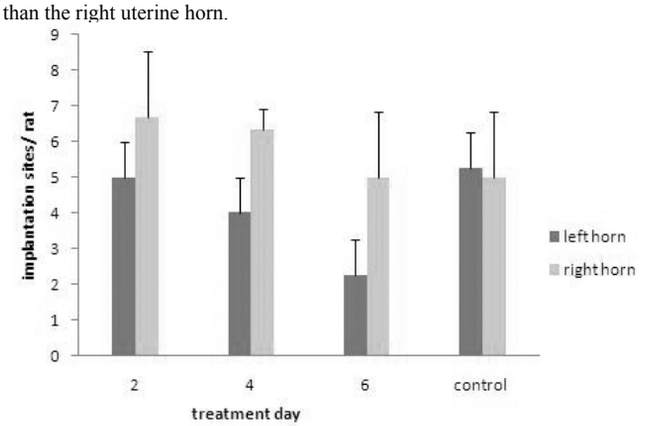


Figure1: AEA-induced decrease in number of implantation sites

CB1 mRNA is markedly increased in placenta and decidua of Day2-treated rats (p=0.019).

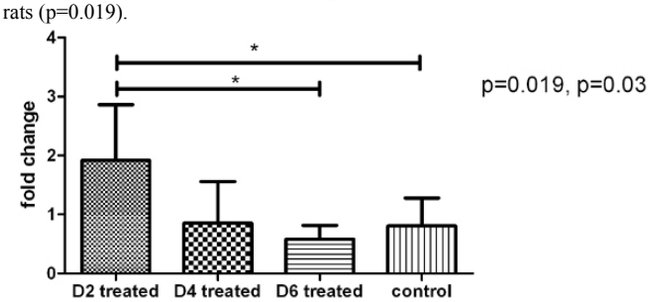


Figure2: Placental CB1 mRNA expression

CB2 mRNA is increased in placenta of Day6-treated rats (p=0.016) and decidua after Day2 treatment (p=0.0099). In decidua of Day4 treated rats, the mRNA of AEA degrading enzyme FAAH (p=0.008) and the TRPV1 receptor (p=0.015) are increased.
Conclusion: Effects of exogenous AEA administration on rat implantation are dependent on the day and site of administration.

F-162

Modulation of the Endocannabinoid System (ECS) in Rats after Exogenous Administration of Anandamide (AEA). Tulay Karasu,[1] Timothy H Marczylo,[1] Bruno M Fonseca,[2] Georgina Correia-da-Silva,[2] Natercia A Teixeira,[2] Justin C Konje.[1] *[1]Endocannabinoid Research Group, Reproductive Sciences, CSMM, University of Leicester, Leicester, United Kingdom; [2]Biochemistry, Faculty of Pharmacology, University of Porto, Porto, Portugal.*
Introduction: Low AEA levels at implantation sites are critical for implantation and early pregnancy maintenance. AEA is a ligand for cannabinoid (CB1, CB2) and vanilloid (TRPV1) receptors and is synthesized and degraded by NAPE-PLD and FAAH, respectively. These receptors and enzymes comprise the ECS. We examined ECS transcript levels after exogenous AEA administration to pregnant rats.
Methods: Pregnant Wistar rats were given AEA (1mg/kg i.p.) or vehicle on Day2, Day4 or Day6 then sacrificed on Day14. ECS transcripts were investigated in decidua, placenta and myometrium of implantation and inter-implantation sites by qRT-PCR. AEA and two congeners (OEA, PEA) were measured by UHPLC-MS/MS.
Results: In AEA-treated rats, NAPE-PLD, TRPV1, CB1 and CB2 transcripts were increased in placenta compared to inter-implantation sites ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.001$) but FAAH was unchanged.

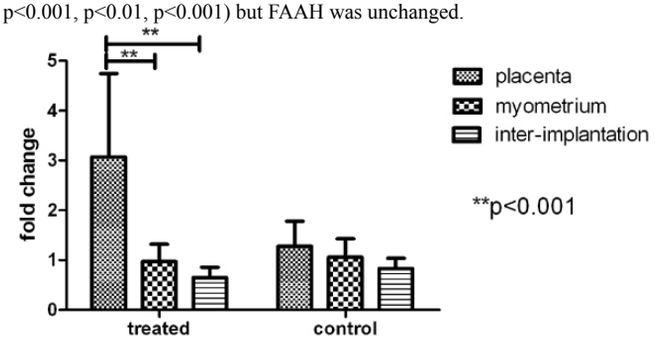


Figure1: NAPE-PLD expression in placenta, myometrium, inter-implantation site

FAAH and CB2 expression in decidua was significantly higher than placenta ($p<0.001$). NAPE-PLD, CB1 and CB2 expression in myometrium were also increased when compared to inter-implantation sites ($p<0.05$, $p<0.001$, $p<0.001$). In control rats FAAH transcript from inter-implantation sites were increased compared to placenta ($p<0.01$). Tissue levels of AEA, OEA and PEA in decidua and placenta were not significantly different. There was a significant correlation between placenta and plasma AEA (p=0.008). D2 treated rats had significantly higher AEA levels compared to D6 treated animals (p=0.011). AEA plasma levels significantly increased with the number of implantation sites (p=0.007).
Conclusion: Exogenous AEA administration modulates the ECS in rat pregnancy. These observations are exciting and suggest that interrupting the ECS may affect implantation.

F-163

The Effect of Exogenous Administration of Anandamide (AEA) on Pregnancy. Tulay Karasu,[1] Timothy H Marczylo,[1] Bruno M Fonseca,[2] Georgina Correia-da-Silva,[2] Natercia A Teixeira,[2] Justin C Konje.[1] *[1]Endocannabinoid Research Group, Reproductive Sciences CSMM, University of Leicester, Leicester, United Kingdom; [2]Biochemistry, Faculty of Pharmacology, University of Porto, Porto, Portugal.*
Introduction: AEA is a lipid molecule that acts via endocannabinoid (CB1, CB2) and endovanilloids (TRPV1) receptor-mediated cell signalling. High uterine AEA levels can lead to failure to implant and to miscarriage. Optimal AEA levels are maintained by regulation of synthesizing (NAPE-PLD) and degrading (FAAH) enzyme levels. Here we investigate the effect of exogenous AEA on fetal resorbtion and changes to CB1, CB2, TRPV1, NAPE-PLD and FAAH transcripts in rat.
Methods: A single injection of AEA (1mg/kg i.p.) or vehicle was given to pregnant Wistar rats on Day2, Day4 or Day6. The rats were sacrificed on Day14, resorbed sites counted and the transcript levels in resorbed sites, decidua and placenta were determined by qRT-PCR.
Results: AEA-treated rats showed more resorbed sites compared to controls.

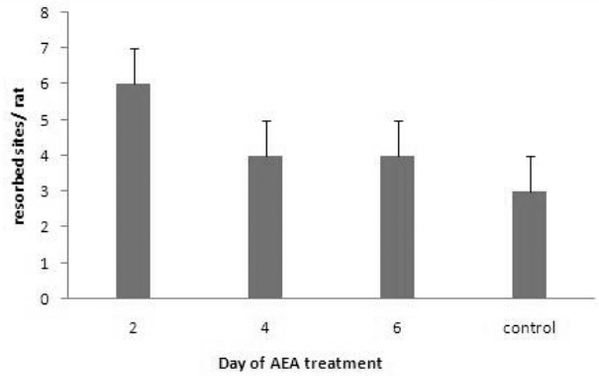


Figure1: AEA induced increase in resorbed sites

Transcript levels of enzymes and receptors in the resorbed sites were similar to decidua. In placenta, NAPE-PLD and TRPV1 transcripts were markedly increased compared to resorbed sites (p=0.0079, p=0.019).

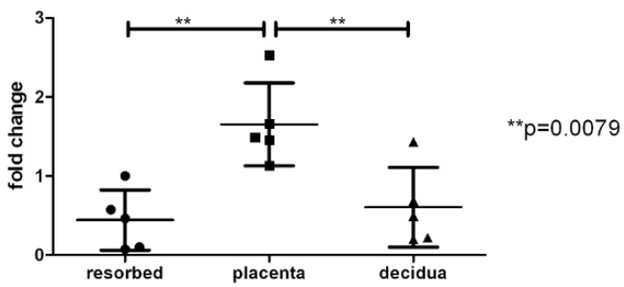


Figure2: NAPE-PLD expression in resorbed sites, placenta, decidua

The expression of FAAH was non-significantly increased in resorbed sites compared to placenta.
Conclusion: Exogenous AEA increases fetal resorbtion in rats.

F-164

The Down-Regulation of LH Receptor in the Rat Uterus. Yoshimitsu Kasahara, Yoshikazu Kitahara, Kazuto Nakamura, Takashi Minegishi. *Obstetrics and Gynecology, Gunma University, Gunma, Japan.*
The luteinizing hormone receptor (LHR) belongs to the family of G protein-coupled receptors that mediate biological effects through cAMP. The expression of this receptor was previously thought to be restricted to gonadal tissue. However, recent studies have shown its presence in many other tissues throughout the reproductive and non-reproductive organs.
We have detected LHR mRNA in the immature rat uterus by Northern blot and down-regulation of this receptor mRNA in the pregnant mare serum gonadotropin (PMSG) - human chronic gonadotropin (hCG) treated immature rats. After administration of hCG, the mRNA levels in the rat uterus declined to a very low level from day 1 to 3 and then rebounded and reached higher than pretreatment values at day 4.
The cultured uterus displayed an hCG concentration-dependent increase in cAMP production in medium, and the immunohistochemical experiment showed that these receptor proteins are expressed in the epithelial cells of endometrium.
These results suggest that functional LHR are present in the immature rat uterus and are down-regulated and up-regulated by signals resulting from hCG treatment.
We think that the uterus model of LH receptor expression might contribute to the investigation of mechanism of LH action in reproductive organs.

Friday

F-165

Progesterone Regulates Estrogen-Induced Transcriptional Responses in Endometrial Stromal Cells: Role of IGF-1. Dustin Manders, Annika Lindqvist, Ruth A Word. *Obstetrics and Gynecology, University of TX Southwestern Medical Center, Dallas, TX, USA.*

ObjectivesE2-induced stromal cell-derived growth factors have been shown to be involved in the pathogenesis of endometrial hyperplasia and endometriosis. Although it has been shown that progesterone downregulates IGF-1R in epithelial cells, the mechanisms by which progesterone protects the endometrium from hyperplastic growth are not entirely clear. Experimental evidence supports the idea that stromal-epithelial interactions may be involved. Here, we used microarray and qPCR analysis of hormone-responsive human endometrial stromal cells treated with vehicle, estradiol (E2, 10 nM), progesterone (P, 100 nM), or E2+P to define the transcriptional profile of these cells in response to steroid hormones. **Results.** Microarray analysis revealed that progesterone, alone or together with E2, resulted in significant downregulation of COUP-TFII (a transcription factor known to control stromal cell aromatase expression and decidualization in vivo) and HOXA10 (an important transcription factor dictating cell fate). Although FGF family members (FGF1, 2,-7, 9, 12, 13, 18 and FGF receptors 1, 2, 3, and 4) were expressed in endometrial stromal cells, none were regulated by E2 or P. Hand2, a stromal-derived transcription factor believed to mediate FGF responsiveness in mouse endometrium, was not expressed in HESCs. EGF, EGF receptor, and EGF response factor 2 were also highly expressed in these cells, but not regulated by E2 and P. In contrast, E2 treatment resulted in significant upregulation of IGF-1 (6-fold, from 1.2 ± 0.1 to 6.5 ± 0.4 U/RPLP0, n = 6 from two cell preps) and P downregulated E2-induced upregulation of IGF-1 significantly (from 6.5 ± 0.4 to 2.5 ± 0.4 U/RPLP0). Further, P resulted in significant loss of ERα in HESCs (from 1.1 ± 0.1 to 0.4 ± 0.1, n = 8 cell preps). Progesterone also resulted in significant decreased expression of CD24 (from 1.2 +/-0.1 to 0.6 +/- 0.03, n = 6 preps), a cell adhesion glycoprotein important in immune cell recruitment. **Conclusions**: These data indicate that progesterone acts to decrease expression of IGF-1 and ERα in endometrial stromal cells and thereby provides new information regarding the mechanisms by which progesterone protects the endometrium from E2-induced hyperplasia. Collectively, these experiments suggest that progesterone-PR interactions in stromal cells protect from E2-induced growth of IGF-responsive endometrial epithelium.

F-166

Increased Hypoxia Inducible Factor-2α Protein Levels in the Endometrium of Women with Heavy Menstrual Bleeding. Jacqueline A Maybin, Donald J Wilson, Hilary OD Critchley. *MRC Centre for Reproductive Health, University of Edinburgh, United Kingdom.*

Significance: Heavy menstrual bleeding (HMB) is a common and debilitating condition for which the underlying pathology remains undefined. Endometrial function in the preceding cycle will impact on subsequent blood loss. Hypoxia inducible factor (HIF)-2 is a transcription factor formed by the dimerization of HIF-2α with a β subunit. HIF-1 is well known as the master regulator of the cellular hypoxic response, but the role of HIF-2 is still to be fully elucidated. Targeted inactivation of HIF-2α in mice revealed defects in vascular remodeling (Pang et al, PNAS, 2000). HIF-2α may have an important role in the significant vascular remodeling that takes place in the human endometrium throughout the menstrual cycle.

Hypothesis: (1) HIF-2α is present in the human endometrium (2) Women with HMB have aberrant HIF-2α compared to those with normal loss.

Methods and Results: Endometrial biopsies were collected from 24 women with ethical approval and consent. All had regular menstrual cycles. Women with endometriosis and fibroids >3cm were excluded. Biopsies were classified as menstrual (n=3), proliferative (n=3), early- (n=3) mid- (n=12) or late- (n=3) secretory based on day of cycle, histological dating and serum ovarian hormone levels on day of biopsy. Nuclear protein extracts were prepared from endometrial tissue from across the menstrual cycle. HIF-2α was detected by Western blot analysis, but only during the early-mid secretory phase. To compare women with normal loss and HMB, mid-secretory samples were collected from women with objectively measured blood loss (n=9). HMB was defined as >80ml and was measured using a modified alkaline-hematin method. Densitometric analysis of Western blots after normalisation to a housekeeping protein, revealed women with HMB had significantly increased endometrial HIF-2α protein detection compared to women with normal loss ($p<0.05$).

Discussion: HIF-2α is present in the human endometrium but only during the early-mid secretory phase. Increased HIF-2α in the mid-secretory endometrium of women with HMB may result in aberrant angiogenesis, leading to increased blood loss during subsequent menses.

F-167

Endometrial Actions of Selective Progesterone Receptor Modulator CDB-4124. Beth McAvey,[1] Liyin Zhu,[2] Satu Kuokkanen,[1] Jeffrey Pollard.[2] *[1]Obstetrics and Gynecology&Women's Health, Montefiore Medical Center, Bronx, NY, USA; [2]Developmental&Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA.*

CDB-4124 (CDB) is a selective progesterone receptor modulator that is in investigational use for symptomatic uterine fibroids and endometriosis. Concerns over negative endometrial effects, specifically hyperplasia, have been raised.

We performed a dose-response of CDB in ovariectomized CD-1 mice.Mice were primed with SQ estradiol (E2) 100 ng and treated with doses of SQ CDB for 3 days: 0.25 mg, 0.5 mg, 1.0 mg, 2.0 mg and 4.0 mg/mouse/day.15 hours(hr) prior to sacrifice,the animals received a 50 ng dose of E2 and uteri were removed 2 hr after receiving bromodeoxyuridine(BrDU).Uteri were weighed,fixed and embedded.Immunohistochemistry using an anti-BrDU antibody was performed to assess DNA synthesis in the endometrial luminal(LE) and glandular epithelium(GE). Cells were counted to 200 cells and labeled nuclei expressed as a % to determine the proliferative index (PI).Control animals were treated using E2 alone, progesterone (P4) alone or CDB alone.

A dose-response was observed for uteri weight and PI in an inverse fashion.

Uteri Weight and Proliferative Index (PI)

	Uteri Weight(grams±SD)	PI(%)
E2 alone	.18±.23	78.4
CDB 0.25	.09±.03	45.4
CDB 0.5	.09±.05	31.4
CDB 1.0	.09±.05	32.6
CDB 2.0	.11±.06	16.6
CDB 4.0	.11±.05	9.3
CDB alone	.05±.01	4.9
P4 alone	.08±.04	0.0

Kruskal-Wallis testing for non-parametric variables revealed a statistically significant difference between the CDB groups for both uterine weight,p= 0.01 and PI,p= <0.001.

Immunohistochemistry was performed to assess amounts of progesterone receptor (PR) and mini-chromosomal maintenance 2(MCM2) protein.Animals were treated as described and sacrificed at 4 hr, 7 hr or 15 hr post-treatment. PR staining in the LE and GE was up-regulated by E2 alone at 4 and 7 hr,however,the PR was down-regulated at15 hr.In a similar pattern,animals treated with CDB 2.0 mg revealed moderate PR staining after 4 hr,with a down-regulation at 15 hr.MCM2 staining was up-regulated by E2 alone and down-regulated by P4 alone in both the LE and GE.Animals treated with CDB had a dramatic decrease in MCM2 staining,similar to that seen with P4. Although we saw a dose-dependent increase in uteri weight after treatment with CDB,a dose-dependent decline in the PI was noted.Furthermore,MCM2 and PR protein staining decreased 15 hr after CDB treatment,suggesting that CDB acts like a progesterone agonist in the LE and GE.

F-168

MFG-E8 (Milk Fat Globule EGF Factor 8 Protein) Is a Novel Endometrial Epithelial Glycoprotein Regulated by Human Chorionic Gonadotropin (hCG) and Secreted Via Microparticles. Abbaa Sarhan,[1] Silvina Bocca,[1] Sandra Anderson,[1] Terry Jacot,[1] Tanya Burch,[2] Julius Nyalwidhe,[2] Claretta Sullivan,[3] Sergio Oehninger.[1] *[1]Dept of OB/GYN, The Jones Institute for Reprod Med; [2]Dept of Microbiology, Leroy T. Canoles Jr. Cancer Research Center; [3]Dept of Surgery, Eastern VA Med School.*

Background: We recently identified MFG-E8 as a novel protein in the human endometrium with predominant localization in epithelial cells, and demonstrated its up-regulation during the window of implantation. MFG-E8 has multiple functions in a variety of extra-uterine tissues related to apoptosis, cell adhesion, neovascularization, and immunomodulation. We hypothesize that MFG-E8 may act as a key modulator of endometrial remodeling and trophoblast invasion.

Objective: Female sex steroids and human chorionic gonadotropin (hCG) play a central role in endometrial development and implantation. The specific aim of this study was to investigate the in vitro regulation of human MFG-E8 transcription, translation, and secretion in human endometrial epithelial cells by estradiol (E_2), progesterone (P4), and hCG.

Methods: Ishikawa cells, used as a surrogate for the endometrial epithelium, were cultured *in vitro* and treated with E_2 and P4 (10^{-8}M - 10^{-6}M), and hCG (500 mIU/ml) for multiple time points. MFG-E8 mRNA expression was determined by real time RT-PCR, and intracellular and secreted MFG-E8 protein detected by immunoblotting. Liquid chromatography mass spectrometry (LC-ESI-MS/MS) was used after trypsin digestion to further characterize the secreted proteins.

Results: No significant difference was found in MFG-E8 mRNA expression after E_2 and/or P4 treatment (at 6-24 h), nor in intracellular MFG-E8 protein after E_2, P4, or hCG treatment (at 24-72 h). On the other hand, hCG significantly increased MFG-E8 secretion (at 72 h; $P<0.05$). Secreted membranous structures, likely microparticles, obtained after ultracentrifugation were visualized with atomic force microscopy ranging in size from ~100 to 200 nm. In addition to the expected 46 kD protein, the microparticles contain a second form of secreted MFG-E8 measuring ~30 kD which was identified and confirmed by MS.
Conclusions: This study demonstrates that hCG, an early embryonic product, stimulates MFG-E8 secretion in association with microparticles in Ishikawa cells. These results strongly suggest that MFG-E8 has the potential to modulate endometrial function and implantation via exocrine and/or paracrine-autocrine effects.

F-169

Alkaline Phosphatase (AP) Discriminates Uterine Epithelium and Decidua during Early Pregnancy. Bibhash C Paria, Wei Lei, Heidi Nguyen, Jeff Reese. *Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA.*
Background: AP activity has been extensively studied in tissues of various species. Although AP is routinely used as a biomarker for liver function and bone formation, its characteristics and relative importance in the uterus in general and during early pregnancy are poorly understood.
Objectives: The objectives of this work in the hamster and mouse were to define: 1) isoforms of AP expressed in uterine cells; 2) species-specificity in steroid hormonal regulation of uterine AP isoform and activity; 3) whether monitoring of uterine AP gene expresssion and activity during early pregnancy possibly could identify the uterine receptive state and stromal transformation to decidua following implantation. The hamster and mouse models were chosen because hormonal necessities for uterine receptivity and implantation differ between these two species. Only P_4-exposed uterus supports implantation in hamsters, similar to humans. In contrast, P_4-treated mouse uterus requires estrogen exposure to initiate implantation.
Methods: A histochemical method was used to localize AP activity in uterine cells. Uterine-specific AP genes were identified by RT-PCR, gene cloning and gene sequencing. Cell-specific AP gene expression was determined by *in situ* hybridization.
Results: Maximum AP activity was noted in the luminal epithelium of hamster diestrous uterus, and mouse proestrous and estrous uteri. Studies involving individual effects of P_4 and E_2 in ovariectomized hamsters and mice showed upregulation of uterine epithelial AP activity by P_4 in hamsters and by E_2 in mice. Histochemical activity, chemical inhibitors, RT-PCR and *in situ* hybridization show that uteri of both hamsters and mice mainly expressed the Akp2 gene that encodes tissue non-specific AP (TNAP). Patterns of *Akp2* mRNA expression and AP activity were analogous to the uterine receptive state and decidua formation following implantation in both species.
Conclusions: Results of this study in two endocrinologically defined species have established that 1) uterine cells of both species mainly expressed the Akp2 gene product TNAP; and 2) uterine receptive state or decidual growth following implantation could be identified by studying AP activity in both species. Collectively our data suggest that uterine TNAP may play a role in differentiation of uterine luminal epithelial cells during the receptive state and stromal cells following implantation. NIH grant RO1HD044741 06A2S1

F-170

Decidualization Alters Vitamin A Signaling in Endometrium. Mary Ellen Pavone, Matthew Dyson, Saurabh Malpani, Toshiyuki Kakinuma, Serdar E Bulun. *Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*
Introduction:
It is known that decidualization alters multiple molecular pathways in endometrium in order to permit successful embryo implantation. We have reported that paracrine factors, including retinoids, secreted from progesterone treated endometrial stromal cells (EIUM) act on nearby epithelial cells to induce the estradiol metabolizing enzyme HSD17B2 . This same induction is not provided by endometriotic stromal cells (OSIS). We have also shown significant differences in retinoid uptake, metabolism and action in endometriotic tissue and stromal cells compared to normal endometrium. Here we further characterize retinoid signaling during decidualization in human stromal cells from EIUM or OSIS.
Material and Methods:
Primary EIUM (n=5) and OSIS (n=5) were isolated, cultured and incubated with a decidualization cocktail of estradiol, medroxyprogesterone, and 8-bromo-cAMP over 2 weeks. Protein and mRNA expression of genes responsible for retinoid metabolism and trafficking including STRA6, CRBP1, ALD1A2, CRABP2, FABP5, RARα, RXRα, PPARβ/δ and CYP26B1 were examined using RT-PCR and western blotting. Decidualization markers including PRL and IGFBP1 were examined using RT-PCR and ELISA. RBP4 secretion was also measured using ELISA.
Results:
Both EIUM and OSIS expressed all intracellular proteins involved in retinoid uptake and metabolism all time points (n=5). Decidualization significantly reduced expression of the genes responsible for RA uptake and shuttling to the nucleus ($p<0.05$). However, mRNA and protein expression of CRBP1, an intracellular carrier protein for retinol, increased, as did mRNA for RBP4, a carrier protein for retinol in the blood which has been described to function in a paracrine manner. Secreted RBP4 was detected by ELISA in the media from decidualized HESC. However in OSIS, there was neither an increase in CRBP1 mRNA nor protein expression with decidualization nor was secreted RBP4 detected.
Conclusions:
RA trafficking in EIUM stroma during decidualization may shift to favor paracrine rather than intacrine signaling, which may enhance signaling to the adjacent epithelium. There is blunting of this signaling in endometriosis. These alterations in retinoid signaling may help explain the decidualization defects, as well as deficient estradiol inactivation (via HSD17B2) seen in endometriosis.
FUNDING: K12HD050121, ASRM Career Development Award (to MEP), 5T32 DK007169 (supporting MD), R37HD038691 (to SEB)

F-171

Uterine Lavage or Aspirate: Which View of the Intrauterine Environment? Natalie Hannan,[1] Guiying Nie,[1] Adam Rainzcuk,[2] Luk Rombauts,[3] Lois Salamonsen.[1] *[1]Uterine Biology, Prince Henry's Institute of Medical Research, Melbourne, Victoria, Australia; [2]Ovarian Cancer Biomarkers, Prince Henry's Institute of Medical Research, Melbourne, Victoria, Australia; [3]Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia.*
BACKGROUND: Fluid within the uterine cavity provides the immediate microenvironment for pre-implantation blastocyst development and the earliest stages of embryo implantation. Recent developments in proteomics, glycomics and lipidomics, now make feasible the analysis of uterine fluid components. Both aspiration and lavage have been used to sample uterine fluid. The similarity or otherwise of the components of samples thus obtained is not known. The aim of this study was to compare proteins in aspirates and lavage samples taken sequentially from the same women. METHODS: Women were subjected to uterine transcervical aspiration (~5-10µl) followed immediately by uterine lavage (5mL) at clinical examination by a single gynaecologist. The samples were analysed by SELDI-TOF-MS, multiplex cytokine and growth factor assays and a specific enzyme activity assay for proprotein convertase (PC)6. The cytokines/growth factors and PC6 had all previously been detected in uterine lavage. RESULTS: While both lavage and aspiration are satisfactory sampling techniques enabling subsequent analysis of uterine fluid components, they are not interchangeable since they provide substantially different protein profiles as examined by three different techniques. While there are many similarities in the overall profiles demonstrated by SELDI-TOF-MS and most of the specific proteins examined were detected in both fluids, their relative abundance did not show significant correlation. CONCLUSIONS: Both aspiration and lavage are appropriate sampling techniques for uterine fluid analysis. However results are neither qualitatively nor quantitatively comparable. A likely explanation is that lavage samples the entire uterine cavity and this includes washing the endometrial surface (glycocalyx), whereas aspiration samples only very locally.

F-172

Endometrial Apolipoprotein Pattern Is Altered in Obese Women but Not in Plasma: Implications in Endometrial Receptivity and Embryo Implantation. Gemma Tamarit,[1] Jose A Horcajadas,[2,3] Jose A Martinez-Conejero,[2] Jose M Arbones,[3] Carlos Simon,[2] Antonio Pellicer.[2] *[1]Obstetrics and Gynecology, Hospital Manises; [2]Fundación IVI-Instituto Universitario IVI, University of Valencia; [3]Arait at I+CS, Hospital Miguel Servet.*
INTRODUCTION
Obese women present lower fertility rates and they have an abnormal lipoprotein profile. It has been found a hCG-dependent down-regulation of apoA-I at mRNA level in the endometrium and that apoA-I is produced by human preimplantation embryos suggesting a possible role in implantation. However, the local regulation at endometrial and systemic level around the window of implantation has not been studied so far.

OBJECTIVE
To investigate the plasma and endometrial lipid profiles of normal and obese women at the time of implantation
METHODS
This prospective study was conducted at the Dr. Peset Hospital and IVI (Valencia, Spain). Four groups of women (n=5 for each) were included: **N1**, normal-weight in natural cycle (BMI<25 kg/m2); **N2**, obese women in natural cycle (BMI>30 kg/m2); **S1**, normal-weight under substitute hormonal cycle; and **S2**, obese women under substitute hormonal cycle. Serum lipids (total cholesterol, HDL, LDL, VLDL, Apo A1, Apo B and Lp(a) and endometrial apolipoproteins (A1, B, CII, CIII and E) were analyzed at days +2 and +7 days after LH peak in natural cycle, and +2 and +7 days after progesterone administration in substitute cycle
RESULTS
Serum lipids and apolipoproteins at +2 and +7 did not show significant variations. At the endometrium, normal-weight women with regular menstrual cycle increased all apos (A1, B, CII, CIII and E) levels at day LH+7 compared to LH+2. This up-regulation was lost in their obese counterparts, who had abnormally high levels at LH+2. Interestingly, the ratio between apolipoprotein (percentage of each apo in total apos) is unchanged except for apo-B. Women with substitute cycles matched the levels of apolipoproteins between normal and obese from day P+2, no significant variations between P+2 and P+7 were observed
CONCLUSION
The present work shows that there is indeed an abnormal protein profile in obese women at the time of implantation that could be involved in endometrial receptivity. However, such regulation is not systemic and it seems to occur at the endometrial level. The augmented local ApoB presence indicates an efflux of LDL cholesterol towards the endometrium. The endometrium could be acting, therefore, as a cholesterol reservoir which may be needed upon embryo implantation.

F-173

Expression of Cytoskeletal-Linking Proteins Radixin, Moesin and Their Phosphorylated Forms in Human Endometrium. Orkun Tan,[1] Kelley Carrick,[2] Jonathan Kim,[1] Orhan Bukulmez,[1] Ann R Word,[1] Bruce R Carr.[1] *[1]Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX, USA; [2]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA.*
Objective: Radixin and moesin belong to ezrin, radixin and moesin (ERM) protein family which are expressed in many different tissues where they play a role in altering cellular structure and form. Our previous study identified ezrin in normal endometrium. Therefore, we hypothesize that radixin and moesin may also be differentially expressed through the endometrium and aid in formation of cell surface structures such as microvilli and pinopodes. Design: Endometrial tissues from reproductive age women (mean age 35±12 years) were obtained from human uteri after hysterectomy conducted for benign diseases other than endometrial disease following institutional review board approval. A total of twenty four endometrial samples from different phases of normal endometrium were studied. Methods: Early proliferative (n = 4), mid proliferative (n = 4), late proliferative (n = 4), early secretory (n = 4), mid secretory (n = 4) and late secretory phase (n = 4) endometrial samples were analyzed for immunoreactive radixin/moesin (ir-radixin/ir-moesin) and their phosphorylated forms (ir-p-radixin/ir-p-moesin) by immunohistochemical analysis. The H-score values were analyzed by one-way ANOVA. Statistical significance was defined as $p<0.05$. Results: Although, ir-radixin and ir-p-radixin were present in the normal endometrium, ir-moesin and ir-p-moesin were absent. In glandular cells, ir-radixin and ir-p-radixin did not vary significantly throughout the cycle. In contrast, stromal ir-radixin and ir-p-radixin were significantly higher in the secretory phase compared to the proliferative phase. Whereas ir-radixin was higher in glandular than stromal cells during the proliferative phase ($p<0.05$), we did not observe any difference in ir-radixin expression between the two cells types during the secretory phase. During the mid secretory phase, ir-radixin and ir-p-radixin were expressed in microvilli and pinopodes. Conclusions: To our knowledge, we are the first to describe the expression of radixin, and p-radixin in normal endometrium. Their presence on apical surfaces of the glandular epithelium, pinopodes, microvilli and stromal cells during the mid and late secretory phases suggest that they may have functional implications for implantation biology.

F-174

Mechanism of Nitric Oxide Induced Down-Regulation of CD55 during Dr-fimbriated *E. Coli* Infection in Human Endometrial Cells. Manu Banadakoppa, David Nowak, Uma Yallampalli, Dan Liebenthal, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
The CD55 (Decay Accelerating Factor, DAF), bound to the plasma membrane protects the cells from autologous complement mediated damage. Many bacteria and viruses utilize CD55 as receptor to invade the cells before establishing infection. Previous work in our laboratory has shown that inhibition of nitric oxide increases maternal mortality in pregnant rats with uterine infection by *E.Coli* expressing the Dr-fimbria, epithelial binding and invasion depends on NO regulated expression of CD55. In the present study we elucidated the mechanism of CD55 expression regulation by NO using Ishikawa endometrial carcinoma cells as an in vitro model of the human endometrial epithelium.
Methods: Ishikawa cells were transiently transfected with a series of deletion constructs of 5'-Upstream Sequence (5'-UPS), 3'-Untranslated region (3'UTR) as well as point mutants of 5'-Upstream Sequence of CD55 containing β-galactosidase reporter gene and then treated with 1mM DETANONOate for 24 h. The effect of NO on CD55 mRNA stability was assessed by arresting transcription using Actinomycin D followed by qPCR to measure the level of CD55 mRNA as a function of time. The half-life of the mRNA was calculated from the decay curve. The HuR mRNA levels in NO exposed cells were evaluated by qPCR.
Results: Upon NO treatment the reporter enzyme activity decreased in cells transfected with 5'-UPS deletion constructs. This decrease was obliterated when the first 108 nucleotide deletion construct was transfected indicating this region as the NO response sequence. This region harbors binding sites for several transcription factors. Among the point mutants the CREB and SP1 binding site mutants did not show a decrease in reporter activity indicating these two trans factors as NO response mediators. The half life of CD55 mRNA in Ishikawa cells was 20 hours and upon NO treatment it was reduced to 10 hours. The 3'-UTR construct in which an AU rich region was deleted showed increased reporter activity demonstrating its CD55 mRNA destabilizing role. HuR protein binds to AU-rich region and maintains the stability of mRNAs. Upon NO treatment the mRNA levels of HuR were decreased which could reduce the stability of CD55 mRNA. Conclusion: The Nitric oxide decreases the CD55 levels by regulating its promoter activity and by reducing the half-life of pre-existing CD55 mRNA.

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Sex Steroid Hormone Effect on Influenza Infection. Sarah Davis, Leigh Sweet, Karen Oppenheimer, Mark Phillippe. *Dept. of Obstetrics, Gynecology & Reproductive Sciences, University of Vermont, Burlington, VT, USA.*
Introduction: Human influenza pandemics have disproportionally affected gravid women, with striking disease severity demonstrated in the second and third trimesters of pregnancy. This study evaluates the effect of 17ß-estradiol (E2) and progesterone on influenza infection in a mouse model.
Methods: 48 ovariectomized (OVX) and 12 cycling CD-1 mice were exposed to isofluorane anesthesia for 6 minutes. Under anesthesia, steroid hormone pellet implantation or sham procedure was performed by subcutaneous technique 14 days after ovariectomy. 21-day continuous release steroid hormone pellets were purchased from Innovative Research of America (IRA) at daily doses of: E2=2µg, low-dose progesterone (LP)=1mg, high-dose progesterone (HP)=1.5mg. Mice were divided into groups: OVX (n=18), cycling (n=12), E2 (n=6), E2+ LP (n=6), LP (n=6), and HP (n=12). Day 3 following implantation, animals were anesthetized with ketamine/xylazine and underwent intranasal inoculation with mouse-adapted H1N1 influenza (strain A/Puerto Rico/8/34 (PR8)) virus at 2500 egg infecting units (EIU). Mice were observed until death or for up to 21 days post inoculation (dpi) for morbidity (weight loss (WL), ruffled fur, or decreased activity).
Results: The mean maximum percent WL for each group was: OVX 23.5% (± 10.9), cycling 19.9% (± 8.8), E2 12.1% (±9.5), E2+LP 15.6% (± 16.6), LP 13.0% (± 12.9), HP 26.8% (± 9.2). The mean between groups was significant for HP vs. E2 (p=0.01), HP vs. LP (p=0.015), HP vs. E2+LP (p=0.046), OVX vs. E2 (0.032), and OVX vs. LP (p=0.047). The mean duration of morbidity for survivors was significantly different between HP and E2, E2+LP, and OVX groups ($p<0.05$) with mean duration (days) for HP 8.3 (±4.2), E2 2.2 (±3.1), E2+LP 2.8 (± 3.8), and OVX 4.0 (± 3.0). No difference was detected for morbidity onset (average 6.5 dpi). While no difference was detected for >20% WL (morbidity) or >30% WL (mortality); 83% of HP animals had >20% and 33% had >30% WL, while E2 had 33% and 0% WL, respectively.
Discussion: Sex steroid hormones affect influenza virus infection morbidity, with HP animals incurring greater WL and longer duration of morbidity.

Interesting trends are observed in morbidity and mortality proxies, with HP and E2 functioning to exacerbate and protect, respectively, during influenza infection. An interesting role for estrogen and progesterone may underlie disease severity demonstrated in gravid women. (Funded by NIH R21HD065396)

F-176

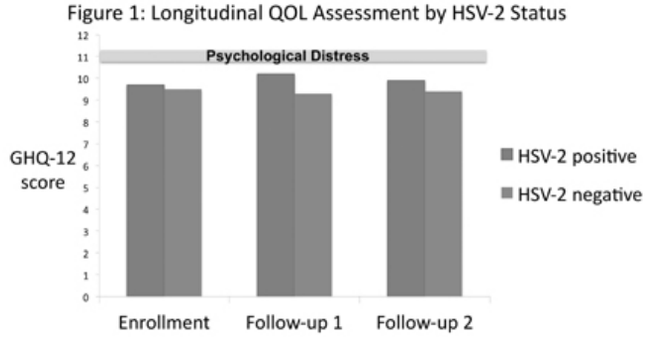
Screening for Herpes Simplex Virus in Pregnancy Does Not Impact Quality of Life. Andrea Edlow,[1] Anjali Kaimal,[1] Hang Lee,[2] Donna Felsenstein,[3] Laura Riley.[1] *[1]Obstetrics and Gynecology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [2]Biostatistics, Massachusetts General Hospital, Boston, MA, USA; [3]Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA.*

Objective: In spite of the potential maternal and neonatal benefits, many object to antenatal HSV screening on the basis of presumed negative psychological impact on pregnant women. Our objective was to evaluate the effect of HSV testing in pregnancy on pregnant women's quality of life (QOL).

Methods: We conducted a prospective cohort study to assess the impact and reliability of HSV serologic assays in pregnancy. Women undergoing antibody testing for HSV were administered the 12-item General Health Questionnaire (GHQ-12), a validated instrument to assess QOL. After receiving results of their serologic testing for HSV-2, QOL was reassessed at 2 subsequent obstetric visits (FU1 and FU2). Chi square and Fisher's exact tests were used to assess the effect of HSV-2 antibody testing on QOL.

Results: 229 patients were enrolled. The prevalence of HSV-2 was 10.5%. Mean QOL for the whole cohort was 9.6 +/- 2.9 at enrollment. Scores ≥11 indicate significant stress. Baseline QOL was not significantly modified by race, age, or genital HSV status, and did not change significantly at FU1 or FU2. Stratifying by HSV-2 status, mean QOL did not change significantly from enrollment to FU1 and FU2, and was not significantly different between groups.

Figure 1: Longitudinal QOL Assessment by HSV-2 Status



Conclusions: The study cohort had a low prevalence of HSV-2. Mean GHQ-12 scores at enrollment, FU1 and FU2 were below the threshold score for significant psychological stress. QOL did not change significantly throughout pregnancy, regardless of receiving HSV-2 positive or negative results.

F-177

Detection of Group B Streptococcus in under One Hour by a Modification of a Previously Described Rapid Diagnostic Test. Jonathan Faro, Gerald Riddle, Karen Bishop, Allan Katz, Sebastian Faro. *Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Science Center at Houston, Houston, TX, USA.*

Introduction: Current screening guidlines for Group B streptococcus (GBS) recommend that all pregnant patients be tested between 35-37 weeks gestation. Traditional culture technique requires 2-3 days before GBS may be identified. Rapid diagnostic tests for GBS have relied on polymerase chain reaction (PCR) technology, and are cumbersome, expensive, and often require specialized training. A rapid test that is inexpensive and easy to perform would allow for intrapartum screening for GBS.

Materials and Methods: Nitrocellulose (NC) membranes were cut into 1.5 cm squares, and then coated with 20 microliters of a 1:40 dilution of a mouse monoclonal antibody against rabbit IgG and allowed to air dry. NC membranes were then blocked with milk diluent and again air dried. Membranes were either next stored at 4 degrees Celsius or inoculated with the sample specimen as follows: A 0.5 McFarland of GBS was set up (GBS was purchased from ATCC), and the bacteria was serial diluted out, starting at 10^6 bacteria per milliliter. To each dilution, a 1:30 dilution of horse-radish peroxidase rabbitt polyclonal antibody against GBS was added to bring the final volume up to 100 microliters. The sample was spun at 3,000 rpm in a microcentrifuge and washed with phosphate buffered saline (PBS) three times. The pellet was resuspended in PBS and 20 microliters was added to the NC membrane. After washing with PBS three times, bound GBS was detected with diaminobenzidiene.

Results: GBS was detected reliably at 10^6-10^2 bacteria per milliliter. Minimal background was observed, and no binding was observed when Enterococcus and Staph aureus were substituted for GBS. All tests were performed in triplicate.

Conclusion: GBS may be detected in as little as 30 minutes by this antibody-based immunoblot, and shows no cross-reactivity with Staph aureus or Enterococcus.

F-178

Synergistic Effects of Bacterial PAMPs on Influenza Infection during Pregnancy. Mark Phillippe, Sarah Davis, Leigh Sweet. *Dept. of Obstetrics, Gynecology & Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT, USA.*

Introduction: Severe human influenza infections are often complicated by concurrent bacterial infections. These studies sought to evaluate the mortality rates (MR) in influenza-infected mice challenged with sublethal dose of bacterial pathogen associated molecular pattern factors (PAMPs); i.e. lipopolysaccharide (LPS) and peptidoglycan (PGN).

Methods: 36 non-pregnant (NP) and 89 timed-pregnant (Pr) CD-1 mice were utilized for these studies. Under low dose ketamine/xylazine, the mice underwent intranasal inoculation (IN) with mouse-adapted H1N1 influenza (strain A/Puerto Rico/8/34 (PR8)) virus at 10,000 egg infectious units (EIU). At 3, 5, 7 or 9 days post inoculation (dpi), the mice underwent intraperitoneal (IP) injections with sublethal doses of LPS or PGN. The mice were observed for term delivery, preterm delivery (PTD), morbidity (determined by ruffled fur, decreased activity and/or weight loss) until death or up to 21 dpi.

Results: For the NP mice, the mortality rate (MR) was 12/16 (75%) occurring on average 10.3 (± 1.7) dpi; for these mice LPS and PGN did not significantly alter the MR. In contrast, for Pr mice with IN on gestational day (GD) 14, the MR was only 1/10 (10%); with addition of LPS (30 μg) on 3 dpi MR = 6/12 (50%) or with PGN (1 mg) MR = 5/10 (50%) (together $p<0.05$). For LPS given 5 and 7 dpi, the MR remained at 3/6 and 3/6 (both 50%); whereas, MR decreased to 1/6 (17%) at 9 dpi. With PGN at 5 dpi, the MR remained 3/6 (50%); whereas, at 7 and 9 dpi the MR decreased to 0/6 and 1/5 (20%), respectively. The GD 14 infected mice delivered within 48 hours when LPS or PGN was given at 3 dpi; i.e. delivery on GD 18-19 consistent with that for untreated control and PR8 alone mice. For the GD 9 infected mice, delivery occurred within 24 hours in 7/8 (86%; $p<0.01$) when LPS was given at 5 dpi (i.e. PTD at GD 15). For the PGN group, PTD occurred within 72 hours in 3/7 (43%) with the remaining 4 delivering on GD 19. For the PR8 alone given on GD 9, the maternal mortality was 2/5 (40%), with the 3 survivors delivering at GD 19-20.

Discussion: These studies have confirmed a significant increase in mortality in pregnant mice from the synergistic effects of influenza infection and exposure to bacterial PAMPs, along with a high rate of PTD when given on GD 14. These adverse outcomes are consistent with those observed in pregnant women afflicted with pandemic influenza. (Funded by NIH R21HD065396)

F-179

Paradoxical Effect of Influenza Infection on Pregnant Mice. Mark Phillippe, Leigh Sweet, Oliver Dienz, Elizabeth Bonney. *Dept. of Obstetrics, Gynecology & Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT, USA.*

Introduction: Severe influenza is associated with higher morbidity and mortality (M+M) in pregnant women compared to non-pregnant. Williams + Mackenzie in 1977 (J Hyg 79:249) described 3x higher mortality among influenza-infected pregnant mice during the 3rd week of gestation. The studies described in this report sought to replicate these observations.

Methods: 45 non-pregnant (NP) and 42 pregnant (Pr) CD-1 mice were utilized. Under low-dose ketamine/xylazine, mice underwent intranasal inoculation (IN) with mouse-adapted H1N1 influenza (A/Puerto Rico/8/34 (PR8)) virus quantified in egg infectious units (EIU). The mice were then observed and weighed daily until death or up to 21 days post inoculation (dpi.). Morbidity was determined by ruffled fur, decreased activity and/or >20% weight loss. The dpi for mortality was recorded. Studies were also performed using this protocol with Pr and NP C57BL/6 inbred mice.

Results: At low inoculation doses (i.e. 6,500-12,500 EIU) morbidity began on 4-7 dpi for Pr and NP mice. At higher-doses (i.e. 50-100, 000 EIU) morbidity began in the 3-5 dpi range for Pr and NP. At the lower-doses, the mortality rates (Table I; (* $p = 0.05$) in the Pr mice were unexpectedly lower than the NP mice (occurred at 7–10 dpi); for the whole lower-dose group Pr mortality (6%) < NP mortality (48%) ($p<0.01$). In the higher-dose ranges, the mortality

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rates (Table I) in the Pr mice (occurred at 9–11 dpi) approached that observed in the NP mice (occurred at 7–9 dpi); for the whole higher-dose group Pr mortality (56%) ≅ NP mortality (80%). This paradoxically lower mortality during pregnancy was also observed among the C57BL/6 mice (i.e. dosed at 5,000 EIU, the mortality in Pr mice = 0/7 (0%) vs. NP mice = 4/7 (57%)).

Discussion: We have been unable to replicate the results described in the 1977 report. Interestingly, for over 30 years no published reports replicated the 1977 report until Chan et al. in 2010 (PLoS One 5:e13757). Overall, these observations suggest that there are yet to be defined unknown variable(s) contributing to the inconsistency in outcome for severe influenza infection in pregnant mice. (Funded by NIH R21HD065396 and NCRR P20 RR021905)

Table 1 (Mortality Rates)

PR8 dose (EIU)	Non-Pregnant	Pregnant
6,500	1/7 (14%)	0/5 (0%)
8,500	4/6 (67%)	1/4 (25%)
10,500	2/6 (33%)	0/4 (0%)
12,500	5/6 (83%) *	0/4 (0%)
50,000	7/7 (100%)	4/8 (50%)
75,000	4/7 (57%)	4/8 (50%)
100,000	5/6 (83%)	6/9 (67%)

F-180

Macrophage Immunosupression as a Pathogenesis of Stillbirth in Lassa Fever. Natalia E Schlabritz-Loutsevitch,[1] GianCarlo Mari,[1] Peter W Nathanielsz,[2] Gene B Hubbard,[3] Igor S Lukashevich.[4] *[1]Obstetrics and Gynecology, University of Tennesse Health Science Center, USA; [2]Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, USA; [3]Pathology, University of Texas Health Science Center at San Antonio, USA; [4]Pharmacology and Toxicology, University of Louisvill, USA.*

Introduction. Maternal undernutrition combined with viral infections during pregnancy is one of the major causes of maternal and fetal mortality in third world countries. An extremely high rate of stillbirth (up to 70%) in the third trimester of pregnancy was reported in Lassa fever (LF). The mechanism behind this phenomenon remains unclear. Maternal and fetal macrophages (MΦ) play an important role in placental development and function. Monocyte/MΦ and dendritic cells are major targets of Lassa virus (LASV). The goal of this study was 1) to evaluate the influence of maternal nutrient restriction on the placental MΦ content (CD68) in a baboon model of maternal nutrient restriction; and, 2) to investigate pro-inflammatory cyto/chemokine responses in monocyte-derived MΦ infected in vitro with LASV. **Material and Methods**. Term placentas (three control and four experimental animals) from a baboon model of maternal nutrient restriction were used in this study. Calculation of the number of CD68 positive cells was performed using a computerized stereology approach. Human monocyte-derived MΦ and HEK293 cells were infected with LASV and non-pathogenic Mopeia virus (MOPV). Pro-inflammatory responses (TNF-alpha, IL-6, IL-8, IP-10) were measured at the level of mRNA expression and protein synthesis. Comparison between the groups was performed using ANOVA and t-Student's test. **Results.** 1) The number of MΦ in stem, intermediate and terminal villi did not differ in undernourished, compared to the control animals ($2.5 \pm 0.7/\mu m^2$ and $2.6 \pm 0.8/\mu m^2$ respectively) 2) LASV infection, but not MOPV infection, resulted in suppression of pro-inflammatory responses. **Conclusion.** Maternal undernutrition itself does not cause changes in the number of fetal MΦ in the placenta at the end of gestation. The LASV-inducible suppressive phenotype can affect MΦ associated innate immune responses resulting in fetal immunodeficiency and fetal loss.

F-181

Can Placental Pathology Findings Predict Mother to Infant Transmission of Genital Mycoplasmas? Alireza A Shamshirsaz,[1] Winston A Campbell,[1] Melinda Sanders,[3] Samadeh Ravangard,[1] Naveed Hussain.[1] *[1]OB/GYN, UCHC; [2]Pediatrics, UCHC; [3]Pathology, UCHC.*

Purpose: Ureaplasma urealyticum (Uu) and Mycoplasma hominis (Mh) are the most common mycoplasma (GM) species that colonize the maternal genital tract. Factors associated with vertical transmission [VT] are not well known and organism identification by culture takes a minimum of 5-7 days, therefore delaying treatment. The aim of this study was to identify placental histopathology that may lead to early identification of infected infants thus facilitating treatment.

Methods: We performed a retrospective study of all maternal and neonatal specimens sent for GM culture between January, 2002 and June, 2010. Maternal cervical cultures were done on all admissions for preterm labor or preterm premature rupture of membranes. Infant tracheal aspirates were done on all intubated infants. Placental histopathology was reviewed by our perinatal pathologist who characterized placental inflammation as inflammation of membranes (IM), chorionic plate (ICP) or umbilical (IU). Data regarding putative perinatal risk factors were extracted. Statistical analysis used Chi square, student "t" test, multiple logistic regression.

Results: Of 807 maternal and 556 infant cultures, 251 were paired maternal-infant samples. Of paired samples, there were 140 positive maternal cultures for GM. There were 37 infants who had a positive culture (giving a VT rate of 26.4%). Significant variables for VT are listed in table. Maternal race, infant gender, multiple gestations, mode of delivery, use of maternal steroids or macrolide antibiotics were not significantly associated with VT. Placental inflammation (IM, ICP, IU) were significantly correlated with VT even after correcting for gestational age, mode of delivery, PPROM, birth weight and duration of rupture of membrane. The best histopathology marker for VT was IOCP (sensitivity 86%, specificity 52%, PPV 40%, NPV 92%).

Table 1. Variables associated with VT transmission and placenta histopathology

Mother-infant trans	GA(wk)*	BW(gm)*	ROM> 12hr*	IM*	ICP *	IU*
Yes (n=37)	26±3	963±449	22 (59%)	32 (86%)	32 (86%)	22 (59%)
No (n=103)	28±3	1162±468	36 (35%)	45 (44%)	48 (47%)	28 (27%)

* $P < 0.01$

Conclusions: Vertical transmission of GM was highly correlated with histopathology evidence of placental inflammation. Since this information is available within 24-48 hrs after birth, it may be helpful in early management strategies to decrease GM associated morbidities in newborn infants.

F-182

Blastocyst Vitrification Is Superior to Slow Freeze Cryopreservation for Frozen-Thawed Embryo Transfer Cycles. Stephanie A Beall,[1] Kevin S Richter,[2] Michael Tucker,[2] Graham James,[2] Michael J Levy,[2] James Segars.[1] *[1]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health; [2]Reproductive Endocrinology and Infertility, Shady Grove Fertility Reproductive Science Center.*

Objective: Based on published literature suggesting vitrification (VIT) was superior to slow freeze (SF), the lab of a large fertility practice transitioned from SF to VIT in January 2009. This study was designed to evaluate clinical outcomes associated with this protocol change.

Design: Retrospective cohort study.

Materials and Methods: Frozen-thawed embryo transfer cycles (FET) cycles performed from Jan 2003 - May 2010 were reviewed. Embryos were obtained from fresh IVF cycles performed in Jan 2002 or later and cryopreserved at the blastocyst stage. Autologous and donor oocyte recipient cycles were evaluated separately. Variables compared between cryopreservation protocols included embryo survival, percentage of blastomeres surviving per embryo, patient age at cryopreservation (for autologous cycles), embryos per transfer, pregnancy by serum hCG, clinical pregnancy (ultrasound confirmation of a gestational sac) and biochemical pregnancy (positive hCG without clinical pregnancy). Comparisons were by X2 or t-test as appropriate.

Results: All treatment outcomes were consistently and substantially better for VIT compared to SF embryos, despite the transfer of significantly fewer vitrified embryos per cycle and slightly but significantly older age at the time of cryopreservation among autologous patients.

		Autologous		Donor Oocyte		
	SF	VIT	p-value	SF	VIT	p-value
Embryos Thawed	5245	563		1470	190	
Post-thaw Survival	88%	92%	0.012	87%	96%	<0.0001
Blastomere Survival	89%	96%	<0.0001	89%	97%	<0.0001
Transfers	2545	305		793	124	
Age at Cryo	33.8	34.3	0.02			
Embryos/ET	1.7	1.6	0.04	1.7	1.5	0.0009
Positive hCG/ET	45%	63%	<0.0001	42%	56%	0.004
Biochemical Preg	25%	12%	<0.0001	28%	20%	0.18
Clinical Preg/ET	34%	55%	<0.0001	30%	44%	0.002

Conclusions: Vitrification was associated with a higher proportion of surviving embryos, an increased proportion of surviving blastomeres per embryo, a relative increase in clinical pregnancy rates per transfer of approximately 50-60% and substantially fewer biochemical pregnancies.

Support: Shady Grove Fertility Center and the National Institutes of Health

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Lower Pregnancy for Donor Oocyte Recipients Compared to Non-Donor Patients Undergoing Frozen-Thaw Embryo Transfer Cycles. Stephanie A Beall,[1] Kevin S Richter,[2] Michael Tucker,[2] Graham James,[2] Michael J Levy,[2] James Segars.[1] *[1]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health; [2]Reproductive Endocrinology and Infertility, Shady Grove Fertility Reproductive Science Center.*

Objective: Compare outcomes of donor oocyte (DO) to non-donor (ND) frozen-thaw embryo transfer (FET) cycles

Design: Retrospective cohort study

Materials and Methods: FET cycles performed from Jan 2003 - May 2010 were reviewed. All embryos were obtained from fresh IVF cycles performed in Jan 2002 or later and frozen at the blastocyst stage on day 5-7. Embryo cryopreservation by slow freezing (SF, performed through Dec 2008) and by vitrification (VIT, from Jan 2009 onward) were evaluated separately. Oocyte donor qualifications included good general health, age 21-31 years, BMI 18-28, and non-smoking.

Results: Regardless of cryopreservation protocol, clinical pregnancy rates per FET were significantly lower for DO compared to ND. With SF, ND vs DO pregnancy rates were 34% vs 30% compared to 47% vs 50% in corresponding fresh transfers from the same embryo cohorts. With VIT, pregnancy rates were 55% vs 44% compared to 24% vs 15% in corresponding fresh transfers from the same cohorts. Among all cycles with cryopreservation of surplus blastocysts during the study period, pregnancy rates were 63% per fresh ND transfer and 69% per fresh DO transfer.

SF				VIT		
	ND	DO	p-value	ND	DO	p-value
Transfers	2545	793		305	124	
Embryos/FET	1.74	1.71	0.23	1.66	1.51	0.014
Clin Preg/FET	34%	30%	0.023	55%	44%	0.038
Embryos/fresh ET	1.92	1.76	<0.0001	1.72	1.56	0.026
Clin Preg/fresh ET	47%	50%	0.20	24%	15%	0.034

Conclusions: Poorer FET outcome among DO recipients was unexpected given the younger age and lack of known fertility problems among oocyte donors. Significantly poorer outcomes of DO vs ND patients among corresponding fresh transfers prior to VIT suggest that embryo cohort quality may have contributed to this trend among VIT cycles. However this cannot account for the same trend among SF FET cycles, as in this case success rates in corresponding fresh cycles were slightly higher with DO. Fresh transfer outcomes between all patients freezing surplus embryos and those actually using them in subsequent FET cycles differed more for DO vs ND patients, thus uterine receptivity may play a greater role in DO than ND failure.

Support: Shady Grove Fertility Center and the National Institutes of Health

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Establishing Serum FSH Threshold To Optimize Controlled Ovarian Hyperstimulation (COH) for Women with Normal Ovarian Reserve: A Pilot Study. Erkan Buyuk, David Kulak, Cheryl Hickmon, Andrew Yu, Harry J Lieman, Sangita K Jindal. *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Women's Health, Albert Einstein College of Medicine, Montefiore's Institute for Reproductive Medicine and Health, Bronx, NY, USA.*

Objective: To determine appropriate serum FSH levels for optimal and non-excessive ovarian response during COH for IVF-ET.

Design: Prospective controlled.

Materials and Methods: Following lupron suppression, daily serum FSH levels were measured during COH in women with normal ovarian reserve, defined as maximum historical FSH≤10 IU/L (n=33). The women were divided by age: Group 1: age<35 (n=10); Group 2: age≥35 (n=23). Women with PCOS were excluded. Student t-test and Mann Whitney U test were used for statistical analyses.

Results: Women in both groups had similar maximum historical FSH and baseline antral follicle count while women in Group 2 had higher body mass index (BMI) ((27.9±5.8 vs. 23.5±3.5, p=0.03) and used more daily recombinant FSH (192±71 vs. 98±95, p=0.002), compared to women in Group 1. Average daily mean serum FSH levels were 11.7±2.9 IU/L for women in Group 1 and 13.7±5.80 IU/L for women in Group 2 (p=0.3). The average number of oocytes retrieved for Group 1 and Group 2 were 18.4±11.8 and 15.5±11.80 p=0.5, respectively. Despite similar FSH serum levels and number of oocytes retrieved, 100% of women in Group1 and only 74% of women in Group 2 had 7 or more oocytes retrieved (p=0.14). In Group 1, the lowest average serum FSH for a cycle that still yielded ≥7 oocytes retrieved was 6.8IU/L. Interestingly in Group 2, women who had <7 oocytes retrieved had greater daily serum FSH levels compared to women whom had ≥7 oocytes retrieved (20±9.5IU/L vs. 12.2±3.3IU/L, p=0.03), despite similar age, maximum historical FSH, antral follicle count and BMI.

Conclusions: A serum FSH level ≥6.8IU/L seems to be sufficient for a proper ovarian response during COH for women with normal ovarian reserve and <35 year old. For women aged ≥35 and normal ovarian reserve, higher serum FSH levels do not necessarily correlate with greater number of oocytes retrieved and may be associated with poorer outcomes in terms of number of oocytes retrieved. Determining serum FSH threshold levels during COH may improve cycle outcome and decrease cost by avoiding unnecessary medication administration.

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Personalized Risk of Multiple Birth after In Vitro Fertilization. Bokyung Choi,[1,2] Benjamin M Lannon,[3,4,5] Michele R Hacker,[4,5] Laura E Dodge,[4] Beth A Melizia,[6] C Brent Barrett,[3,4,5] Wing H Wong,[1,7] Mylene WM Yao,[1] Alan S Penzias.[3,4,5] *[1]Research and Development, Univfy Inc., Palo Alto, CA, USA; [2]Applied Physics, Stanford University, Stanford, CA, USA; [3]Reproductive Endocrinology & Infertility, Boston IVF, Waltham, MA, USA; [4]Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston, MA, USA; [5]Obstetrics, Gynecology, and Reproductive Biology, Harvard Medical School, Boston, MA, USA; [6]Reproductive Endocrinology & Infertility, Alabama Fertility Specialists, Birmingham, AL, USA; [7]Statistics, Stanford University, Stanford, CA, USA.*

Background

Multiple births account for nearly one-third of deliveries following in vitro fertilization and remain a significant cause of morbidity associated with assisted reproduction. Large-scale implementation of elective single embryo transfer to reduce the multiple birth rate has been challenging. Further, patient-specific adaptations of national guidelines on embryo transfer have not been reported. We describe an approach to develop and validate a patient-specific prediction model that analyzes the influence of patient-, embryo- and clinic-specific factors to predict the personalized probability of multiple birth.

Methods

We applied boosted tree methods to analyze IVF treatment cycles in which the transfer of 2 embryos resulted in single or multiple live births from a data source comprising over 33,000 IVF treatment cycles . There were 2,413 independent cycles used to develop the model. Validated results were compared to control models.

Results

The prediction model showed a 146% improvement over the age-based control in its ability to predict the multiple birth probabilities, with estimated standard errors of 4.0% to 8.4%, and improved discrimination among different prognoses by 16.0%. Multiple birth probabilities predicted by the model were significantly different from control models in over half of the patients with a dynamic range of 11.8% to 54.8%, compared to only three discrete probabilities generated by the age-based control model.

Conclusions

We showed that IVF patients have inherently different risks of multiple birth that can be predicted, even when only two embryos were transferred. The use of clinic-, patient- and treatment-specific prediction models of the risk of multiple birth may provide an evidence-based method to counsel patients.

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Thrombotic Stroke in Early Pregnancy Associated with Ovarian Hyperstimulation Rescued by Thrombolectomy and Revascularization of a Large Vessel Occlusion. Radiation Risks and Outcome. Natalie Chua,[1] Wendy Teoh,[1] Robert L Brent,[2] Wickly Lee,[3] Seong-Feei Loh,[1] Jerry Chan.[1,4,5] *[1]Reproductive Medicine, KK Women's and Children's Hospital, Singapore; [2]Medicine, Thomas Jefferson University, Philadelphia, USA; [3]Radiology, Tan Tock Seng Hospital, Singapore; [4]Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School, Singapore; [5]Experimental Fetal Medicine Group, Yong Loo Lin School of Medicine, NUS, Singapore.*

Severe Ovarian Hyperstimulation Syndrome (OHSS) affects 2-4% of IVF patients, which may rarely result in thrombotic stroke secondary to a hypercoagulable state. Here we report a case where timely thrombolectomy led to improvements in clinical outcome with a normal live birth.

A 38 year old woman with tubal disease underwent a long protocol IVF-ICSI cycle at KKIVF centre with lucrin down-regulation, follitropin-α ovarian stimulation and hCG trigger. Following double-embryo transfer and hCG luteal phase support, she developed early onset severe ovarian hyperstimulation syndrome (OHSS) with significant ascites and haemoconcentration four days

post embryo-transfer. She was admitted for intravenous fluid therapy and abdominal drainage of ascites. Additionally, thromboembolic stockings and low-molecular weight heparin prophylaxis were initiated. Despite resolution of haemoconcentration, she developed a thrombo-embolic middle cerebral artery stroke which was confirmed on magnetic resonance angiography 16 days post embryo-transfer. She was screen negative for thrombophilia and had no carotid stenosis nor cardiac or deep venous thrombus. She had a positive pregnancy test at that point in time, and because of the abdominal drain, thrombolytic therapy was not attempted.

A thrombolectomy revascularisation of large vessel occlusion (TREVO) procedure under fluoroscopic imaging successfully removed the internal carotid artery clot, with neurological improvements. Radiation exposure was calculated at an upper limit of 111 mGray of radiation at 18 days post fertilisation, below the 200 mGray threshold for inducing structural malformations and miscarriages. The couple opted for continuation of pregnancy, with the delivery of a live born male fetus in good health.

Timely thrombolectomy in thrombotic stroke may be a safe and effective treatment option in pregnant women with OHSS complicated by thrombotic stroke. Treatment of OHSS induced Strokes and early pregnancy radiation exposure issues will be discussed.

F-187

The Impact of Obesity and Infertility Treatment on Pregnancy Outcomes: The Colorado State Birth Registry Cohort. Isiah D Harris, Evan Carey, Dina Itani, Sahar Stephens, William Schlaff. *Obstetrics and Gynecology, University of Colorado, Denver, Aurora, CO, USA.*

Objective: To determine how BMI impacts cesarean section rates (and other pregnancy related morbidities) in pregnancies resulting from in vitro fertilization (IVF) as compared to spontaneous pregnancies.

Methods: A secondary data analysis was performed on all couples who delivered in the Colorado Birth Records Database from 2007-2009. Inclusion criteria were any deliveries recorded registry from 2007-2009. Exclusion criteria were deliveries for which there was missing data regarding type of delivery, BMI or other identified covariates. Initial descriptive statistics were performed and then a multivariate Poisson regression model was constructed to assess the risks of BMI for spontaneous pregnancies versus IVF pregnancies.

Results: 211,735 patients met the inclusion criteria. Of those, 45,461 were excluded for missing data. 163,805 spontaneous pregnancies and 2,469 pregnancies resulting from IVF were analyzed. The IVF cohort was older, wealthier, and more likely to have private insurance. Cesarean section (19.5% vs 52.8%), NICU admissions (6.3% vs 25.3%), and preterm birth rates (8.8% vs 35.3%) were all higher in the IVF cohort compared to the spontaneous pregnancy cohort. After multivariate analysis, the risk ratios of c-section rate, preterm birth, and NICU admission associated with obesity were all higher in obese women in the IVF pregnancy cohort (see table 1).

Unadjusted Risk Ratios Stratified by Mode of Conception

	BMI Category	Spontaneous Pregnancies	p-value	IVF Pregnancies	p-value
		n = 163,805		n = 2,469	
Cesarean Section					
	Underweight	0.88	0.177	0.91	<0.001
	Normal	1.0	--	1.0	--
	Overweight	1.01	0.866	1.19	<0.001
	Obese	1.09	0.073	1.49	<0.001
Preterm Delivery					
	Underweight	1.16	0.131	1.29	<0.001
	Normal	1.0	--	1.0	--
	Overweight	1.03	0.630	1.06	0.002
	Obese	0.99	0.900	1.21	<0.001
NICU Admission					
	Underweight	1.12	0.398	1.08	0.006
	Normal	1.0	--	1.0	--
	Overweight	1.01	0.866	1.19	<0.001
	Obese	1.09	0.073	1.49	<0.001

Excludes those with previous c-section.

Conclusion: While obesity was associated with a higher risk of cesarean delivery, preterm delivery, and NICU admission in all patients, and the impact of obesity was greater in patients conceiving via IVF than those conceiving spontaneously. A BMI greater than 30 acts exacerbates the pregnancy related risks of undergoing IVF. Patients should be carefully counseled and consideration given to restricting IVF for obese patients.

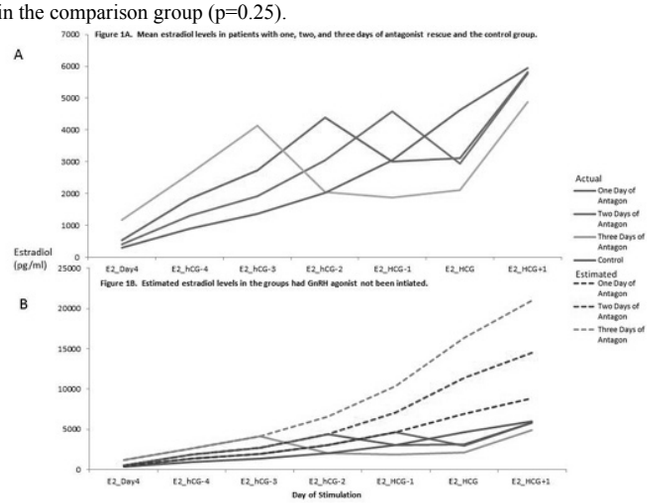
F-188

GnRH Antagonist Rescue Reduces Serum Estradiol in High Responders at Risk for OHSS While Maintaining Excellent ART Outcomes. Micah J Hill,[1,2] Rebecca J Chason,[1,2] Mark D Payson,[1] James H Segars,[2] John M Csokmay.[1] *[1]OBGYN, Walter Reed Army Medical Center, Washington, DC, USA; [2]Program in Reproductive and Adult Endocrinology, NICHD, Bethesda, MD, USA.*

Objective: GnRH antagonist rescue is designed to allow assisted reproductive technology (ART) patients at high risk of ovarian hyperstimulation syndrome (OHSS) to continue to oocyte retrieval without having cycle cancellation. The objective of this study was to evaluate the effect of GnRH antagonist rescue on serum estradiol and ART cycle outcome.

Methods: This was a retrospective cohort study at a U.S. military-based ART program. ART patients on a GnRH agonist for pituitary suppression with evidence of ovarian hyper-response had the GnRH agonist discontinued and a GnRH antagonist initiated for 1-3 days. Exogenous gonadotropin stimulation was continued until follicle sizes reached 18-20mm. 387 patients underwent GnRH antagonist rescue and a comparison group of 271 patients with estradiol levels over 4000 pg/ml on the day of hCG was selected. Estimated estradiol slopes were generated based on the slope of estradiol rise prior to antagonist rescue.

Results: GnRH antagonist rescue decreased the mean estradiol level by 35% on the first day of use (Fig 1A). Peak estradiol levels were decreased by 35-77% from estimated peak levels had antagonist rescue not been performed (Fig 1B). There was no difference in cycle cancellation for severe OHSS risk between the antagonist rescue and control groups (1.5% versus 1.1%, p=0.74). There was no difference in oocyte maturity rate (82% versus 83%, p=0.78) or fertilization rate (69% versus 67%, p =0.15) between the antagonist rescue and comparison groups, respectively. The percentage of high grade embryos on day three and the blastocyst development rate were also similar between groups. The live birth rate was 41.8% in the antagonist rescue group and 36.9% in the comparison group (p=0.25).



Conclusion: GnRH antagonist rescue reduced serum estradiol levels and enabled cycle completion with high live birth rates in patients at risk for OHSS. GnRH antagonist was associated with high oocyte quality, blastocyst development, and pregnancy outcome.

F-189

Correlation between Anti-Mullerian Hormone (AMH) and IVF Outcomes in Patients with Elevated Basal FSH Level. Jack Yu Jen Huang, Tomer Singer, Hung-Chin Liu, Zev Rosenwaks. *The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College-New York Presbyterian Hospital, New York, NY, USA.*

Objective: Anti-Mullerian hormone (AMH) has been shown to be more accurate than FSH as a predictor of ovarian reserve. The study objective is to determine if correlation exists between basal AMH level and IVF outcomes in patients with elevated basal FSH level (>11mIU/ml).

Design: Retrospective analysis

Materials and Methods: The study cohort was comprised of 282 patients who underwent IVF treatment from 4/2008 to 3/2010. Serum FSH and AMH samples were drawn before starting IVF treatment. AMH was measured using AMH Gen II ELISA immunoassay. Patients underwent standard controlled ovarian hyperstimulation. HCG was administered when 2 follicles attained 17 mm. Retrieval occurred 35 hours later. ICSI was performed if indicated. Correlations

between AMH, FSH, maternal age, IVF outcomes were analyzed by Pearson correlation coefficient (r), ANOVA, and receiver operator characteristics (ROC)/area under the curve (AUC).
Results: Correlation between AMH level and oocyte yield is stronger (r=0.44, P<0.001) than that between FSH and maternal age and oocyte yield (r=-0.31, P<0.001, and -0.15, P<0.05 respectively). Using ROC analysis, AMH cut-off of 0.6ng/ml rendered 74% sensitivity and 64% specificity in predicting low oocyte yield (<4) (AUC=0.73, 95% CI=0.7-0.8, P<0.0001). Among patients with elevated basal FSH>11.1mIU/ml, those with AMH>0.5ng/ml had significantly higher peak estradiol (E2) level, number of eggs retrieved, and clinical pregnancy rate compared to patients with AMH<0.5ng/ml.

AMH and IVF outcomes among patients with basal FSH>11.1mIU/ml

	AMH<0.5	AMH>0.5	P values
Peak E2 ±SD (ng/ml)	1027.6±607.9	1756.6±824.4	0.0005
No. eggs retrieved	5.1±3.0	9.5±4.1	0.0007
Clinical pregnancy/transfer %	29.5	64.3	0.04

Conclusions: Among patients with elevated basal FSH, AMH level is helpful in predicting successful IVF outcomes. These findings need to be confirmed in larger prospective study.

F-190

Innovation of Digitalized Microfluidic System with Electrowetting-on-Dielectric (EWOD) Chip Used in In Vitro Embryo Culture System. Hong-Yuan Huang,[1,2] Da-Jeng Yao,[3] Hung-Ju Huang,[2] Shih-Kang Fan,[4] Chin-Jung Li,[1] Ming-Yi Lee.[5] *[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Kwei-Shan, Tao-Yuan, Taiwan; [2]Obstetrics and Gynecology, Chang Gung University and College of Medicine, Kwei-Shan, Tao-Yuan, Taiwan; [3]Nanoengineering and Microsystem, National Tsing-Hua University, Hsin-Chu, Hsin-Chu, Taiwan; [4]Materials Science and Engineering, National Chiao Tung University, Hsin-Chu, Hsin-Chu, Taiwan; [5]Graduate Institute of Medical Mechatronics, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan.*
Introduction: Microfluidic microchannel system for IVF is considered to provide more in vivo-mimicking environments to enhance the preimplantation embryo development in embryo culture system. EWOD is a microfluidic strategy that exploits surface tension as a means of manipulating liquid droplets at current stage. In this study, we aimed to develop an innovated EWOD digitalized microfluidic system that is capable of transporting dynamic medium droplets with mammalian embryos in culture system.
Material and methods: EWOD digitalized microfluidic system with SiO2 dielectric layer containing defined electrode patterns and conducting wires will be investigated to study the basic function for manipulating the microdrop with different cultured medium as well as biosamples (gametes and embryo) to mimic the Fallopian tube environment in a droplet. To further study the EWOD digitalized microfluidic system for in vitro cultured embryo; female ICR mice were stimulated by i.p. injection of 10 IU PMSG and injected 48 h later with 10 IU HCG. Female mice were mated with male mice of the same strain, mice were killed by cervical dislocation 22 h post-HCG administration. Cumulus-free zygotes were gently transferred to EWOP chip or traditional culture dishes containing microdrops of HTF medium covered with warm light white mineral oil in a tissue culture incubator. Embryos were checked to monitor cleavage.
Results: We have successfully manipulate the function of medium droplet containing bio-sample (motile sperm, oocytes, zygote and cleavage embryo) in EWOD chip with modified complex dielectric layer and voltage in culture incubator. The devolvement of cultured embryo in EWOD system is also compared to traditional culture system.
Conclusions: EWOD digitalized microfluidic system are capable of culturing mammalian embryos in a microfluidic biological manner.

F-191

Economic Factors & Decisions To Freeze Excess Embryos. Emily Jungheim,[1] Lisa Pollack,[2] Kenan Omurtag,[1] George Macones,[1] Randall Odem,[1] Barton Hamilton.[2] *[1]Obstetrics & Gynecology, Washington University, St. Louis, MO, USA; [2]Olin School of Business, Washington University, St. Louis, MO, USA.*
Background & Objective: Cryopreservation of excess embryos is important to the application of safe, cost-effective ART. Desipite this, little is known regarding the impact of ART insurance coverage on decisions to freeze excess embryos. Our goal was to investigate associations between ART insurance coverage and consent to embryo cryopreservation.
Methods: This was an IRB-approved retrospective study of women undergoing their first ART cycle between '01-'08 at our institution. Women without an embryo transfer and those using donor oocytes were excluded. 1043 women qualified. Billing and medical records were reviewed for patient ART insurance status, age, address, history of prior live birth, and consent to embryo cryopreservation (signed prior to ART). Home value was used as a surrogate marker of patient socioeconomic status (SES) and was obtained from a public database providing home value estimates, Zillow.com. Associations between variables and consent to embryo cryopreservation were assessed with standard bivariate statistics followed by multivariate logistic regression and stratified analyses to control for potential confounding. Analyses were done in SPSS.
Results: In bivariate analyses, ART insurance coverage (RR=0.89,CI=0.85-0.93) and history of prior live birth (RR=0.93,CI=0.87-0.99) were negatively associated with consent to embryo cryopreservation, while home value was positively associated with it (p<0.001). In multivariate logistic regression all variables remained significant. When stratified by ART insurance coverage, stronger associations were seen between SES and consent to embryo cryopreservation among women without coverage. History of prior live birth was no longer significant.

TABLE: Adjusted odds ratio (aOR) for factors associated with consent to embryo cryopreservation

Women with ART insurance coverage		
Variable	**aOR**	**95% CI**
Home Value ≤ $146,500 (reference)	---	---
Home Value $146,501-$237,000	1.865	0.927-3.752
Home Value ≥ $237,001	3.702	1.445-9.482
History of prior live birth	0.584	0.302-1.126
Women without ART insurance coverage		
Home Value ≤ $146,500 (reference)	---	---
Home Value $146,501-$237,000	3.78	1.356-10.473
Home Value ≥ $237,001	4.136	1.563-10.946
History of prior live birth	0.534	0.220-1.293

Conclusions: This is the first work demonstrating negative associations between ART insurance coverage and consent to embryo cryopreservation. ART insurance coverage often excludes embryo cryopreservation. Future work will determine if changes in ART insurance policy will promote embryo cryopreservation.
Support:K12HD063086

F-192

The Optimum Number of Oocytes Needed To Predict a Live Birth Outcome Depends on Maternal Age, FSH Level, and BMI. Anupama SQ Kathiresan,[1] Yenisel Cruz-Almeida,[2] Rebekah Valthaty,[1] David I Hoffman,[3] Wayne Maxson,[3] Marcelo J Barrionuevo,[3] Vanessa N Weitzman,[3] Daniel R Christie,[3] Gene F Manko,[3] Steven J Ory.[3] *[1]Department of Obstetrics and Gynecology, University of Miami, Miami, FL, USA; [2]Research Services, University of Miami, Miami, FL, USA; [3]IVF Florida Reproductive Associates, IVF Florida Reproductive Associates, Margate, FL, USA.*
Objective:
During in vitro fertilization (IVF) cycles, a low number of retrieved oocytes (RO) is generally associated with poor pregnancy outcomes. To our knowledge, no studies have been done investigating the number of RO needed to optimize chances of live birth (LB) based on maternal age, follicle stimulating hormone (FSH) level, and body mass index (BMI). The objective of this study is to determine the number of RO needed to predict LB in specific age, FSH level, and BMI groups.
Materials and Methods:
A retrospective analysis of 5263 IVF cycles done at a major infertility center between 2004 and 2010 was performed. Inclusion criteria for this study included a first non-donor cycle resulting in fresh embryos transferred on day 3 or day 5. Cycles were grouped based on specific age, FSH level, and BMI groups. A hierarchical linear regression analysis was performed to determine what patient characteristics were significantly predictive of a higher number of RO. Receiver operating characteristic (ROC) curves were then used to determine the number of RO that was predictive of LB for each group.
Results:
Of the 1662 cycles that fulfilled the inclusion criteria, 606 cycles (36.5%) resulted in LB. A hierarchical linear regression demonstrated that maternal age, FSH levels, and BMI were significantly predictive of a higher number of RO (p<0.001). The optimal number of RO predictive of LB for the overall cohort was 8 oocytes (p<0.001). The following RO thresholds were statistically significant: for age groups <35, 35-37, and 38-40, the RO thresholds were 9 (p=0.005), 8 (p<0.001), and 7 (p<0.001) oocytes; for FSH groups <10, 10-14.9, and >20 IU/L, the RO thresholds were 8 (p<0.001), 8 (p=0.002), and 4 (p=0.016); for BMI groups 18.5-24.9 and 25-29.9 kg/m^2, the RO thresholds were 8 (p<0.001) and 5 (p=0.012), respectively.

Friday

Conclusions:
The optimal number of RO that is predictive of LB is dependent on maternal age, FSH level, and BMI. Therefore, retrieving the highest number of oocytes for all women undergoing IVF may not always be the ideal strategy.

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Do Outbred Mice Represent a Better Model for Human Embryos in Quality Control Assays for IVF? Zaraq Khan, Heather S Wolff, Jolene R Fredrickson, David L Walker, Elizabeth A Stewart, Dean E Morbeck. *Division of Reproductive Endocrinology & Infertility, Department of OB/GYN, Mayo Clinic, Rochester, MN, USA.*
OBJECTIVE: Optimizing *in vitro* fertilization (IVF) outcomes depends on minimizing stress to embryos in the laboratory. This study is designed to determine if outbred mice are more sensitive than other strains to *in vitro* stress as measured by cell number in cultured blastocysts.
DESIGN: Laboratory experiment comparing response of 4 mouse strains to 3 adverse culture conditions.
MATERIALS AND METHODS: Fresh one-cell embryos from outbred (CF1), inbred (FVB), B6/CBA hybrid (F1) and frozen hybrid embryos from EmbryoTech™ (F2) were compared in a standard mouse embryo assay (MEA) using six doses of each of three *in vitro* stressors. Embryos were cultured in microdrops of Global media without protein with the following: (A) 0 to 10 µM cumene peroxide in mineral oil, (B) 0 to 0.002% TX-100 in culture media, and (C) 270 to 370 mOsm culture media. Expanded blastocysts (EB) at 96 hours were fixed and stained with immunohistochemical dye TOPRO-3. Embryos were analyzed and cell numbers were counted with an LSM 510 confocal microscope (Zeiss, Jena, Germany) utilizing 3D imagery. All studies were conducted in triplicate ; data were analyzed with ANOVA and reported as mean ± SD.
RESULTS: CF1 embryos detected cumene peroxide and TX-100 at concentrations >50% lower than other strains tested as evident by decreasing cell numbers in the inner cell mass (ICM).

Effect of Cumeme Peroxide Across Mouse Strains

Cumene Peroxide	FVB	F1	F2	CF1
0µM	15(45)	27(59)	18(68)	20(43)
2µM	13(44)	21(59)	20(36)	18(39)
4µM	12(33)	26(42)	18(49)	14(29)
6µM	14(32)	24(42)	17(42)	7(23)
8µM	7(24)	22(40)	16(36)	0
10µM	0	15(38)	0	0

ICM(trophectoderm)

Effect of TX-100 Across Mouse Strains

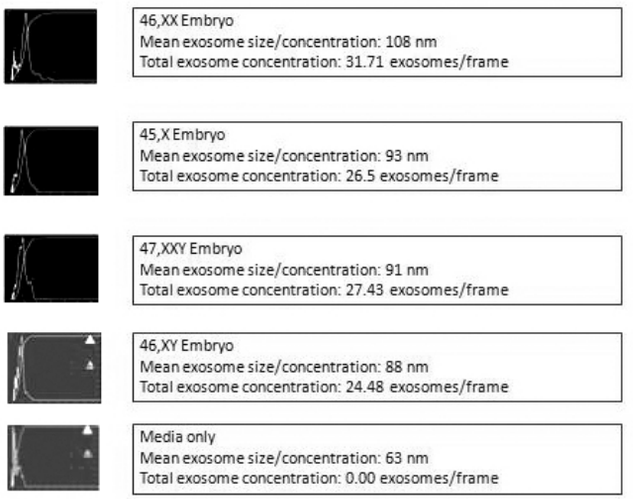
TX-100	FVB	F1	F2	CF1
0%	13(38)	17(42)	20(41)	17(40)
0.001%	14(33)	13(31)	21(51)	16(28)
0.0013%	14(27)	16(32)	24(47)	13(22)
0.0015%	16(23)	15(20)	21(34)	9(18)
0.0017%	8(24)	13(24)	18(25)	6(13)
0.002%	11(13)	12(19)	13(25)	0

ICM(trophectoderm)

No difference in strains was noted for changes in osmolality.
CONCLUSIONS: CF1 embryos are genetically complex and diverse (like human embryos) and are more sensitive to toxins than either inbred or hybrid mouse embryos. Further research is warranted to determine if the current practice of using F2 hybrid embryos is optimal for research and quality control for human IVF.
SUPPORT: Mayo Clinic, Rochester, Department of OB/GYN research fund.

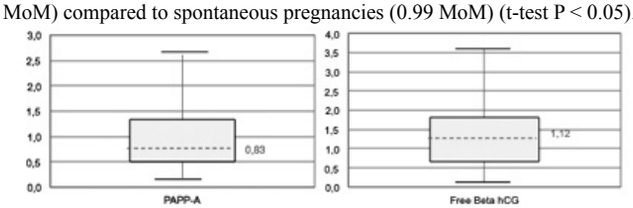
F-194

Exosome Content in Blastocyst Culture Medium from Embryos with Different Karyotypes. Miriam S Krause, Steven T Nakajima, Henry C Bohler, Greg L Christensen, Cicek Gercel-Taylor, Douglas D Taylor. *Obstetrics and Gynecology, University of Louisville, Louisville, KY, USA.*
Hypothesis: Culture medium from blastocysts obtained from patients undergoing in vitro fertilization (IVF) exhibits different exosome patterns based on different karyotypes, as determined by preimplantation genetic screening (PGS).
Background: Exosomes are small membrane vesicles secreted by various cells and are important for information exchange between cells, delivery of functional RNA to other cells, antigen presentation and cell adhesion. Exosomes released by the blastocyst may be an important mediator for implantation.
Methods: Culture medium from day 3 blastocyst culture of patients undergoing routine IVF protocol at our institution and desiring PGS (preimplantation genetic screening) was examined for the exosome content. Aliquots were run on agarose-based gel columns and then analyzed for absorbance to identify the exosome-containing fractions. The pooled exosome fractions were applied to the NanoSight LM10 (NanoSight, Wiltshire, UK), and individual exosome profiles were analyzed. The exosome profiles of blastocysts with different karyotypes were compared.
Results: Culture medium from n=4 blastocyst cultures has been examined so far with 4 primary karyotypes. The exosome patterns were noted to be distinctly different in 46XX versus 46 XY, 45X or 47XXY embryos and a negative control. Mean exosome size/concentration varied from 88 to 108 nm. Total exosome concentration ranged from 24.5 to 31.7 exosomes/frame.
Discussion: Different exosome patterns secreted from various karyotype blastocysts could lead to altered cell to cell signalling and possibly explain the increased risk of miscarriage with abnormal karyotypes. Sample collection is ongoing to confirm these findings.



F-195

Evaluation of PAPP-A and Free β-hCG for the Screening of Down Syndrome in Egg Donation Pregnancies. Valeria Savasi,[1] Arianna Laoreti,[1] Luca Mandia,[1] Luciano Ghisoni,[1,2] Piergiorgio Duca,[3] Irene Cetin.[1] *[1]Department of Clinical Sciences L Sacco, Unit of Obstetrics and Gynecology, University of Milan, Italy; [2]Bi-tech, Bi-tech Ltd, Italy; [3]Institutes of Medical Statistics and Biometry, University of Milan, Italy.*
Since the first successful use of donated oocytes in 1984, oocyte donation has become an integral part of modern assisted reproductive care, resulting in good pregnancy rates and the birth of healthy babies. Prenatal diagnosis in egg donation pregnancies addresses specific issues, due to the separation between the "oocyte age"of the conceptus and the "uterine compartment age" of the mother. First trimester screening for Down syndrome combines measurement of nuchal translucency and fetoplacental markers, free β-hCG and pregnancy-associated plasma protein-A (PAPP-A). The aim of this study was to undertake an analysis of serum maternal analytes results in singleton pregnancies conceived using egg donation and naturally conceived pregnancies. We compared PAPP-A and free β-hCG multiples of median (MoM) values of 158 normal egg donation singleton pregnancies versus MoM values of 435 matched spontaneous pregnancies. Exclusion criteria were multiple gestations, structural fetal malformations and chromosomal abnormalities.
Overall, significantly lower PAPP-A levels were detected in oocyte donation pregnancies (0.83 MoM) than in controls (1.02 MoM) (t-test $P < 0.001$). Free β-hCG levels were significantly higher in oocyte donation pregnancies (1.12 MoM) compared to spontaneous pregnancies (0.99 MoM) (t-test $P < 0.05$).



Free β-hCG and PAPP-A levels in egg donation pregnancies.

Egg donation pregnancies have reduced PAPP-A levels and increased free β-hCG levels. These results may be a consequence of the hormonal treatment used to establish endometrial receptivity. Nevertheless, it is still to be determined the role of the interaction between the elderly uterine compartment and the embryo, which is immunogenetically unrelated to the mother. Adjustment of first trimester serum markers levels may be considered to reduce the likelihood of receiving false-positive results and the rate of invasive diagnostic procedures.

F-196

Current Cycle Characteristics Predict Pregnancy Outcome in Patients Undergoing Single Embryo Transfer. Gary Levy,[1] Kevin Richter,[2] Micah J Hill,[1] Eric Widra,[2] James Segars.[1] *[1]Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Health, Bethesda, MD, USA; [2]Reproductive Endocrinology and Infertility, Shady Grove Fertility Center, Rockville, MD, USA.*

Objective: To examine in-cycle characteristics that predicted clinical pregnancy in patients undergoing single embryo transfer (SET).

Design: Retrospective cohort study.

Materials and Methods: Patients that underwent fresh, autologous single embryo transfer ART cycles between January 2004 and December 2010 were included in the analysis. Clinical pregnancy was the primary outcome variable and was defined as an intrauterine gestation on ultrasound examination at 6-8 weeks. Age, BMI, infertility diagnosis, cycle type (pituitary down-regulation), total gonadotropin dose, number of oocytes retrieved, use of assisted hatching, peak estradiol levels, number of frozen embryos available at cycle completion, prior full-term delivery, and ethnicity were evaluated. Logistic regression was used to determine predictive in-cycle variables correlating with SET pregnancy.

Results: One thousand nine hundred and eighty-seven patients underwent fresh, autologous, single embryo transfer ART cycles. The pregnancy rate for all fresh, non-donor ART SET cycles was 63%. Clinical pregnancy rates declined significantly and gradually with increasing age and BMI. Age 20- <25(71%), 25- <30(67%), 30-<35(65%), 35-<40(58%), 40+(45%) (p<0.001). BMI <30(65%) vs > 30(51%) (p=0.017). Multiple regression analysis demonstrated that clinical pregnancy rate was associated with increasing number of frozen embryos available at cycle completion (p=<0.0005) and history of prior full term delivery (71%) vs (52%) (p=<0.0005). Increasing total gonadotropin administered was correlated with a decreased pregnancy rate (p=<0.0005).

Conclusion: Non-donor, in-cycle ART characteristics correlated with SET outcome and may be useful in counseling patients at the time of embryo transfer. While age and BMI were significantly associated with pregnancy success, no cutoffs were identified where SET would not be recommended.

F-197

Pregnancy Outcomes in Multiparous Women 40 Years and Older with Spontaneous, IVF Autologous Oocyte and Donor Oocyte Singleton Pregnancies. Stephanie Lin,[1] Tomer Singer,[2] Elizabeth Milbank,[1] Nora Ward,[1] Daniel Skupski,[1] Amos Grunebaum.[1] *[1]Obstetrics and Gynecology, NewYork Presbyterian Hospital Weill Cornell Medical College; [2]Center for Reproductive Medicine and Infertility, Weill Cornell Medical College.*

OBJECTIVE: To compare neonatal and maternal outcomes in multiparous patients ≥ 40 years of age with spontaneous, IVF autologous oocyte (IVF AO) and IVF donor oocyte (IVF DO)singleton pregnancies .

STUDY DESIGN: Retrospective cohort study including all deliveries in women ≥40 years of age from 2008-2010 at our institution. Exclusions included multiple gestation and nulliparity. Mode of delivery, gestational age at delivery, NICU admission, birth weight, apgar scores, neonatal length of stay and the rates of hypertensive disorders of pregnancy (HDP) and gestational diabetes were recorded.

RESULTS: 795 pregnancies out of 15,637 deliveries met inclusion criteria. There were 642 spontaneous conceptions, 92 IVF AO, and 61 IVF DO pregnancies. Those with spontaneous conceptions and IVF AO pregnancies were younger but had a higher gravidity and parity. Those with IVFDO pregnancies had a shorter mean gestational age at delivery (268 vs. 272 days, p=.004) but there was no difference in birthweight, gender, or the rates of preterm delivery or small for gestational age. There was no difference in Apgar scores or in the rates of induction, gestational diabetes, or NICU/CCN admissions. Those with IVF DO and AO pregnancies were more likely to undergo cesarean (68.9% IVFDO vs 59% IVFAO vs. 43.5% p<.0001). In patients with a history of a prior vaginal birth, patients with IVF DO pregnancies were more likely to undergo a primary cesarean (OR 3.2 95%CI 1.7 to 6.0 p < .001) than those with spontaneous or IVF AO pregnancies. IVF donor oocyte pregnancies were at increased risk of developing a hypertensive disorder of pregnancy compared to spontaneous conceptions and IVFAO (OR 8.2 95%CI 2.2 to 35.6 p=.002).

CONCLUSION: Multiparous women ≥40 years of age with IVF AO and IVF DO pregnancies have similar neonatal outcomes to those who conceive spontaneously. Women with IVF DO pregnancies are at increased risk of cesarean delivery even after a prior successful vaginal delivery and of developing a hypertensive disorder of pregnancy. These findings can help aid in counseling older patients with IVF AO and IVF DO pregnancies.

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Neonatal and Maternal Outcomes in Nulliparous Women 40 Years or Older with Spontaneous, IVF Autologous Oocyte or IVF Donor Oocyte Singleton Pregnancies. Stephanie Lin,[1] Tomer Singer,[2] Elizabeth Milbank,[1] Nora Ward,[1] Daniel Skupski,[1] Amos Grunebaum.[1] *[1]Obstetrics and Gynecology, NewYork Presbyterian Hospital Weill Cornell Medical College; [2]Center for Reproductive Medicine and Infertility, Weill Cornell Medical College.*

OBJECTIVE: To compare neonatal and maternal outcomes in nulliparous patients 40 years or older with spontaneous, IVF autologous oocyte (AO) and IVF donor oocyte (DO) singleton pregnancies.

STUDY DESIGN: Retrospective cohort study including all deliveries in women 40 years of age or older during 2008-2010 at our institution. Exclusions included multiple gestation and patients with prior delivery. Mode of delivery, gestational age at delivery, NICU admission, birth weight, apgar scores, maternal length of stay and the rates of hypertensive disorders of pregnancy (HDP) and gestational diabetes (GDM) were recorded.

RESULTS: 572 patients met inclusion criteria out of 15,637 deliveries during 2008-2010 (316 spontaneous pregnancies, 153 IVF AO pregnancies and 103 IVF DO pregnancies). Those with IVF DO pregnancies were significantly older than spontaneous and IVF AO pregnancies. Table 1 shows comparisons of neonatal and maternal outcomes. Patients with IVF DO pregnancies are at increased risk of cesarean section, preterm birth, HDP and neonatal NICU admission. There was no difference in apgar scores, birth weight, rate of small for gestational age infants and GDM.

Outcomes

	Spontaneous (316)	IVF AO (153)	IVF DO (103)	OR 95%CI	P
Age	41.5±1.4	41.8±1.4	45.5±2.8		<.0001(β,γ)
Cesarean Section	174 (55.1%)	87 (56.9%)	83 (80.6%)	4.9(2.5 to 10.1)	<.0001 (β,γ)
Preterm Birth	25 (7.9%)	17 (11.1%)	18 (17.5%)	2.6(1.1 to 5.7)	.02(β)
Hypertensive Disorder	27 (8.5%)	19 (12.4%)	18 (17.5%)	2.3 (1 to 4.9)	.04(β)
Gestational Diabetes	27 (8.5%)	19 (12.4%)	18 (17.5%)		.58
NICU/CCN	21 (6.7%)	15 (9.8%)	16 (15.5%)	2.7 (1.1 to 6.1)	.02(β)
Birth Weight	3284±562	3242±659	3193±652		.46
Small for Gestational Age (<10%)	38 (12%)	19 (12.4%)	8 (7.8%)		.44
Maternal Stay Postpartum	3.2±1.3	3.3±1.8	3.9±1.4		<.0001(β,γ)

CONCLUSION: Nulliparous women ≥40 with IVF DO pregnancies are more likely to deliver via cesarean, have a baby admitted to the NICU, and develop a HDP compared to women with spontaneous or IVF AO pregnancies. These findings can aid in counseling older women with IVF DO pregnancies.

F-199

Neonatal and Maternal Outcomes in Women 40 Years and Older with IVF Autologous Oocyte and IVF Donor Oocyte Twin Pregnancies. Stephanie N Lin,[1] Tomer Singer,[2] Daniel Lee,[1] Stephanie Purisch,[1] Daniel Skupski,[1] Amos Grunebaum.[1] *[1]Obstetrics and Gynecology, NewYork Presbyterian Hospital Weill Cornell Medical College; [2]Center for Reproductive Medicine and Infertility, Weill Cornell Medical College.*

OBJECTIVE: To compare neonatal and maternal outcomes of IVF autologous oocyte (IVF AO) and IVF donor oocyte (IVF DO) twin gestations in patients ≥ 40 years of age.

STUDY DESIGN: Retrospective cohort study. All deliveries in women ≥40 years of age from 2007-2010 at our institution were included. Spontaneous conceptions, singleton gestation, triplet gestations or fetal demise of both twins were excluded. Mode of delivery, length of hospital stay after delivery, gestational age at delivery, NICU admission, birth weight, apgar scores and rate of gestational diabetes (GDM) and hypertensive disorders of pregnancy (HDP) were recorded.

Outcomes

	IVF AO N=74	IVF DO N=63	OR(95%CI)	P
Age	41.2±1.4	44.3±2.7		<.001
Gestational Age at Delivery	252±18	253±14		.83
Length of Stay Postpartum	3.9±1.0	4.7±1.9		.003
Cesarean Section Rate	65 (87.8%)	61 (96.8%)	4.2(.8 to 20.3)	.06
Preterm Birth <32 weeks	7 (9.5%)	3 (4.8%)	.5 (.1 to 1.9)	.34
Induction	4 (5.4%)	6 (9.5%)	1.8 (.5 to 6.8)	.51
HDP	11 (14.9%)	24 (38.1%)	3.5 (1.6 to 8)	.003
GDM	7 (9.5%)	10 (15.9%)	1.8 (.64 to 5.1)	.3
NICU/CCN	61 (41.2%)	58 (46%)	1.2 (.75 to 2)	.46
Baby A Weight (grams)	2427±536	2616±534		.05
Baby B Weight	2388±557	2471±516		.33

RESULTS: During 2007-2010 there were 20,394 deliveries. There were 906 twin gestations and 137 met inclusion criteria. Of these, 74 were IVF AO pregnancies and 63 were IVF DO pregnancies. Table 1 compares outcomes for IVF AO and IVF DO pregnancies. Patients with IVF DO pregnancies were older, had a longer hospital stay and had higher risk of HDP. There was no difference in rate of cesarean section, rate of preterm birth, rate of NICU/CCN admission, apgar scores, birth weight, and GDM.

CONCLUSION: Women 40 years of age and older with twin gestations who conceive with donor oocytes are at similar risk for preterm birth, cesarean section, and NICU/CCN admission compared to those who conceive with autologous oocytes. IVF DO pregnancies are at increased risk of developing HDP and having a longer hospital stay. It is unclear if this increased risk of HDP is related to interactions between the pregnancy and the mother or is mainly attributable to the increased age of these patients. Further studies are needed in a larger cohort of patients.

F-200

The Role of Ovulation Induction and IUI in Those with Unilateral Tubal Occlusion: A Conservative Approach. Alan M Martinez,[1] Isela M Robertshaw,[2] Julie M Sroga,[1] Ilana B Ressler,[1] Michael A Thomas,[1] Stephen R Lindheim.[1] *[1]OB/GYN, University of Cincinnati, Cincinnati, OH, USA; [2]OB/GYN, TriHealth, Cincinnati, OH, USA.*

Objective: HSG is the accepted standard to diagnose tubal patency. In contrast to bilateral tubal occlusion where therapy is directed towards laparoscopic correction or IVF, the treatment of unilateral tubal occlusion (UTO) is less clear including conservative OI and IUI directed towards the patent tube. The aim of our study was to assess the value of OI-IUI and pregnancy outcomes in those with UTO.

Design: Retrospective case controlled review.

Methods: We evaluated patients diagnosed on HSG with UTO (n=17) (proximal [n=5] and mid-distal or distal occlusion [n=12]) from 2007 to 2011. Inclusion included women <38 years; regular menstrual cycles; normal sperm parameters; and findings on HSG of normal spill from 1 fallopian tube. Patients in the control group underwent donor insemination (n=72) with the same inclusion criteria except for bilateral tubal spill. Treatment included observation or OI-IUI with Clomiphene Citrate. All cycles were monitored by ultrasound; hCG given when lead follicle >18mm (unless recruited follicle on obstructed side); and IUI performed 24-36 hours later. Primary outcomes measured were demographics and clinical pregnancy (CP). Chi Square and ANOVA were used to compare groups.

Results: Baseline demographics including age (32.2±4[±SD] vs 33.4±2 yrs, p-NS) and BMI (27.9±7 vs 28.2±8kg/m2, p-NS) were similar between UTO and control groups. Between HSG and treatment, spontaneous pregnancy occurred in 4(33%) of women with UTO (1 proximal-3 distal), and 0(0%) in the control group. After OI-IUI, CP rates were similar (46%,n=6/13) and 24%, n=17/72, p-NS) for UTO and control groups, however, CP/cycle were significantly higher with UTO (20%,6/30 and 7.3%,17/233,p=0.048). Overall, CP occurred in 1(20%) and 9(75%) with proximal and distal UTO, respectively. Twenty-four (80%) cycles recruited a dominant follicle on patent side resulting in a 25% CP/cycle, in contrast no pregnancies occurred (0%,0/6) if recruitment occurred on side of UTO (p=0.3).

Conclusions: While tubal spasm may account for false (+) HSG findings, it is less likely with distal occlusion. Pregnancy rates are not compromised in women with UTO and conservative treatment with OI-IUI appears justified as a first line approach obviating more aggressive therapies including LSC and IVF.

F-201

A Thickened Endometrial Stripe on Day 2 of Estrogen-Priming Protocols Does Not Affect IVF Outcomes. Evelyn Mok-Lin, Anate Aelion Brauer, Owen K Davis, Zev Rosenwaks. *Center for Reproductive Medicine and Infertility, Weill Cornell Medical College, New York, NY, USA.*

OBJECTIVE: It has been shown that a thickened baseline endometrial stripe (ES) in GnRH agonist down-regulated IVF cycles results in lower pregnancy rates. Patients on late-luteal estrogen priming protocols often present with a thickened ES on day 2, and their cycles may be delayed for this reason. However, the significance of a thickened ES in estrogen priming protocols has not yet been reported. The objective is to compare IVF outcomes in women who began ovarian stimulation with a thickened ES to those with an ES ≤5mm.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Patients who underwent late-luteal estrogen priming for IVF between 1/1/2005-10/31/10 were identified. Patients with a delayed start date, progesterone >1ng/ml or cysts on day 2 were excluded. Patients with an intention for cryopreservation upfront were excluded. Controlled ovarian hyperstimulation (COH) with FSH and hMG was performed in a standard protocol. HCG was administered when there were at least 2 follicles ≥17mm and retrieval was performed 35 hours later. The main outcome measures were pregnancy, ongoing/delivered and failed pregnancy rates. Other outcomes included: units of gonadotropins required, peak E2 level, cancellation rate, number of oocytes retrieved and number of embryos transferred. Statistical analyses included $\chi 2$ and Mann-Whitney tests. $P<0.05$ was deemed statistically significant.

RESULTS: 572 IVF cycles met criteria; 445 cycles were started on day 2 with an ES ≤5mm (mean 3.9, range 2-5mm) and 127 cycles were started on day 2 with an ES >5mm (mean 7.3, range 5.1-12.9mm). There were no significant differences between the two groups in terms of age, BMI, previous IVF cycles, baseline FSH/LH/E2 levels, total units of gonadotropins, peak E2 level, cancellation rate, number of oocytes retrieved or number of embryos transferred. There were no significant differences in pregnancy, ongoing/delivered or failed pregnancy rates. A secondary analysis comparing cycles started with ES <5mm, 5-7mm and >7mm also showed no significant differences in pregnancy outcomes.

CONCLUSIONS: A thickened ES on day 2 following estrogen priming does not affect IVF outcomes. These patients should begin COH on day 2 without a need for delay.

F-202

Antral Follicle Count Measurement in Oocyte Donors Is Not Associated with Recipient IVF Outcomes. Vasiliki A Moragianni,[1,2] Ariel Mullen,[2] Alan S Penzias,[1,2] Brian M Berger.[1,2] *[1]Obstetrics & Gynecology, Division of Reproductive Endocrinology & Infertility, Beth Israel Deaconess Medical Center, Harvard Medical School; [2]Reproductive Endocrinology & Infertility, Boston IVF.*

OBJECTIVE

Antral follicle count (AFC) is considered a reliable, non-invasive measure of ovarian reserve. However, little data exists on the predictive value of this test in oocyte donors (OD). We designed this study to evaluate the effect of OD AFC on recipient IVF outcomes.

DESIGN

Retrospective cohort study.

MATERIALS AND METHODS

In our institution, all OD's are screened with cycle day 2-4 AFC. We reviewed the charts of all OD cycles performed between 2008 and 2010. We collected data on donor and recipient demographics and cycle outcomes. We classified patients in 4 AFC quartiles and the primary outcome measure was clinical pregnancy. We utilized a one-way ANOVA to compare continuous and chi-square to compare categorical variables across AFC categories.

RESULTS

A total of 190 OD's met inclusion criteria and were stratified into one of the 4 AFC categories. Baseline characteristics were statistically similar across AFC strata, as were fertilization and clinical pregnancy rates.

Table 1. IVF outcomes

			AFC		
	10-15	16-21	22-27	>27	P-value
n	51	49	47	43	
Age (yr)	25.02 ± 2.76	25.31 ± 3.02	25.02 ± 2.95	25.63 ± 2.20	0.69
Body mass index	22.81 ± 3.01	23.44 ± 3.06	22.92 ± 2.51	22.76 ± 2.80	0.64
Antal follicle count	12.92 ± 1.80	18.35 ± 1.89	24.32 ± 1.85	36.74 ± 8.36	<0.01
Peak Estradiol (pg/ml)	2716.00 ± 855.62 a	2858.97 ± 978.56 a	2927.85 ± 963.55 a	3571.61 ± 1252.09 b	<0.01
No. of oocytes retrieved	16.94 ± 7.36	19.67 ± 6.99	18.62 ± 8.20	20.79 ± 6.76	0.07
No. of embryos fertilized	11.08 ± 5.09	12.37 ± 5.09	12.32 ± 6.38	13.79 ± 4.42	0.11
Fertilization rate	0.67 ± 0.18	0.64 ± 0.19	0.66 ± 0.19	0.68 ± 0.16	0.31
No. of embryos transferred	2.00 ± 0.20	2.02 ±0.25	2.00 ± 0.51	1.88 ± 0.32	0.22
No. of embryos frozen	2.47 ± 3.49 a,b	4.25 ± 4.51 a	4.66 ± 4.31 a	5.93 ± 4.28 b	<0.01
Clincal pregnancy rate	30 (58.52)	32 (65.31)	26 (55.32)	24 (55.81)	0.74

Data presented as mean ± SD, or n (%). Values with the same letter do not differ statistically.

CONCLUSIONS

Baseline OD AFC is not associated with fertilization or clinical pregnancy rate of recipients. Larger, prospective studies can further assess its efficacy as a predictor of recipient IVF outcomes.

F-203

Encapsulation of Endometrial Cells for Embryo Culture. Maria Mundi,[1] Jose Serna,[1] Jose A Horcajadas.[2] *[1]IVI-Zaragoza, IVI (Instituto Valenciano de Infertilidad), Zaragoza, Spain; [2]Araid at I+CS, Hospital Miguel Servet, Zaragoza, Spain.*

INTRODUCTION

Although culture media for human embryo development have rich high technical properties, embryo co-culture with endometrial cells monolayer adds benefits such as nutrients, embryotrophic substrates, growth factors, cytokines, and helps detoxifying the culture medium removing metabolites. Establishing co-culture system has disadvantages as cost, microbial contamination and difficulties in the availability of endometrial cells monolayer, have let IVF laboratories to switch to sequential media techniques.

OBJECTIVE

To develop a new, simple, inexpensive, and easy way to store epithelial endometrial cells encapsulated in a 3D matrix that can be cryopreserved ready to use in embryo culture media as a newly coculture technique.

METHODS

Early luteal phase endometrial biopsies were performed after oocyte pickup in donors. Epithelial cells were isolated and cultured until a confluent cell monolayer were achieved. Cells were resuspended in DMEM 1.5% (w/V) low viscosity sodium alginate. The encapsulation system for other cells type was published in 2008 (Bressel et al., 2008). Briefly, it is a compressor that forces the solution to flow with the aid of a peristaltic pump. Drooping into a CaCl2 solution, alginate microcapsules were developed. Microcapsules were kept in the culture media to check their viability before freezing procedure.

RESULTS AND DISCUSSION

Several endometrial biopsies were processes as described and viability of endometrial cells was demonstrated. This system offers the possibility of developing a system to encapsulate endometrial cells of patients who will undergo IVF treatment offering a possibly better way to culture their embryos.

F-204

Profiling of GnRH-Antagonist (ant) Cycles: Predicting the Confusing Nature of Serum Estradiol. Ilana B Ressler, Sofia Mirkopoulos, Julie M Sroga, Alan M Martinez, Michael A Thomas, Steven R Lindheim. *OB/GYN, University of Cincinnati, Cincinnati, OH, USA.*

Objective: GnRH-ant used in a flexible regimen facilitates shorter and simpler treatments compared to GnRH-a IVF cycles. It is underutilized, however, in part due to the confusing nature of a decline or plateau of serum estradiol (E2) following GnRH-ant administration. Our goal was to profile these cycles to predict how individuals will respond to GnRH-ant.

Design: Retrospective chart review.

Materials & **Methods:** Charts were reviewed from patients undergoing both autologous IVF (n=74) and oocyte donation (n=157) from 2007 to 2011. OI was achieved using rFSH (75-600 IU daily). GnRH-ant was begun when lead follicles were 12-13mm with an additional 75 IU of rFSH/day. Main outcome measures were serum E2 patterns following GnRH-ant, which were categorized as a progressive rise (Group 1), decline (Group 2) or plateau (<10% rise or fall) (Group 3). Regression model was used to assess the effects of age, BMI, diagnosis and ovarian reserve markers (AMH, AFC and day 3 FSH/E2) as model variables for effects on serum E2. Secondary measures included clinical pregnancy outcomes.

Results: A decline or plateau of serum E2 was seen in 37% of cycles following GnRH administration. Specifically, autologous cycles resulted in a decline and plateau in 12% and 8% and donor 22% and 17%, respectively. Regression analysis showed a higher AFC (p=0.02) and AMH (p<0.01) were predictive of a decline in E2. Age, BMI and diagnosis were not predictors of E2 pattern.

Patient Characteristics

	Normal Rise (n=147)	Decline (n=46)	Plateau (n=38)	p-value
Mean (±SD) Age	29.4±6.6	27.7±4.9	29.4±5.7	NS
Mean BMI	24.4±5.3	22.8±2.4	23.8±3.7	NS
Mean AFC	15.2±7.0	18.3±8.7	14.2±6.8	0.02
Mean AMH	2.3±2.1	6.7±9.3	2.9±1.9	<0.01
Mean FSH	6.4±5.5	6.0±2.9	6.7±3.0	NS
Mean E2	25.7±30.2	25.4±23.0	33.2±41.0	NS

Two patterns of E2 drop and plateau were noted: early (<cycle day 10, n=16) and late (≥cycle day 10, n=68). An early pattern was associated with higher AFC (p=0.04), while a late pattern was associated with a lower peak E2 (p<0.01). No differences in pregnancy rates were noted in autologous (Group 1-28%; Group 2-50%; Group 3-33%, p-NS) and oocyte donor cycles (Group 1-71%; Group 2-70%; Group 3-64%, p-NS).

Conclusion: E2 decline and plateau is a frequent occurrence in GnRH-ant cycles, though they do not compromise clinical pregnancy outcomes. Ovarian reserve markers including AMH and AFC appear to be the only markers that predict the E2 pattern.

F-205

Insights from Gestational Surrogates (GS) Carrying for Gay Male Couples Undergoing Assisted Reproduction. Ilana B Ressler,[1] Danielle Bessett,[2] Julie M Sroga,[1] Sarah Rompola,[2] Michael A Thomas,[1] Steven R Lindheim.[1] *[1]OB/GYN, University of Cincinnati, Cincinnati, OH, USA; [2]Sociology, University of Cincinnati, Cincinnati, OH, USA.*

Objective: To gain insight into the experiences of GS for gay male couples using assisted reproductive technology (ART) and report areas of needed improvement.

Design: Questionnaire-based cohort study.

Materials & **Methods:** A self-administered questionnaire was sent to GS (n=51) who have previously or are currently assisting gay male couples.

Results: To date 10 (20%) questionnaires have been returned. The average age was 37.8 years (range 34-48) and all were identified through a GS Agency. Eight GS (80%) have successfully delivered (6 singletons and 5 twins) for gay male couples and 2 women (20%) were pregnant at the time of completing the questionnaire. Ninety percent of the women were heterosexual, 80% married and all have had at least one child of their own. Altruistic motives were cited as the most common reason for becoming a GS, and 20% stated finances as primary motivation. Compensation for services averaged $28,000 (range $5,200-30,000) and 80% received additional gifts. None had any reservations regarding carrying for a gay couple, and 20% reported a preference to carry for a homosexual couple. All GS disclosed the couples' sexual orientation to friends and 90% to their extended families. Despite comprehensive discussion by the GS agency and IVF Program, women reflected that they actually did not fully understand the legalities as the process unfolded. Four (40%) GS reported a lack of support from the IVF Program. The number of visits by the intended parents prior to delivery ranged from 0 to "countless." Despite enormous ante- and postpartum support from family and intended parents, and denying difficulty with separating from the infant(s), 2 (25%) reported postpartum depression. Six (60%) reported they would agree to be a GS for the same couple again, however, only 38% of gay male couples polled desired to use the same GS.

Conclusion: GS who agree to carry for gay male couples are comfortable with their choice. Despite extensive educational efforts by the GS agency and IVF Program, confusion still exists. In addition, emotional support throughout the entire process, particularly the postpartum period, is essential.

F-206

Clinical Validation for Repeat Ovarian Reserve Screening (ORS) with the Clomiphene Citrate Challenge Test (CCCT) in Returning Fertility Patients. Isela M Robertshaw, Mazen E Abdallah, Glen E Hofmann. *Obstetrics and Gynecology, Bethesda Center for Reproductive Health and Infertility, Cincinnati, OH, USA.*

Objective: To determine the clinical relevance of a repeat ORS (CCCT) testing.

Design: Retrospective chart review.

Methods: Patients who had initial ORS (CCCT) upon return were offered repeat testing. Values of FSH on either day 3 or 10 in excess of 12 mIU/mL

Friday

were indicative of a high incidence of a poor response to hMG, or greater than 14.5 mIU/mL, consistent with diminished ovarian reserve.
Results: Ninety nine patients had repeat CCCT. Sixty nine (69.7%) had normal CCCT on both examinations, 17 had a reassuring CCCT on initial screening converting to one predictive of a poor response to hMG (17.2%). 19 (19.3%) convert from a normal CCCT to an abnormal CCCT, of which 16 had an FSH >12mIU/mL on initial screening (84%). 11 (11%) had abnormal CCCT on both occasions. No patient with an abnormal CCCT on initial screening had a repeat normal CCCT.
Conclusions: Thirty six women who had a NL initial CCCT (40.9%) had a repeat CCCT indicative of a poorer prognosis. Repeat ORS is indicated in returning fertility patients. A significant number will have screening indicative of a need for more aggressive management, and an equal number convert to an abnormal CCCT requiring counseling on diminished ovarian reserve and possible ovum donation. Patients with an abnormal CCCT initially do not warrant repeat testing.

F-207

Anti Mullerian Hormone Correlates with Embryo Morphology in IVF Cycles. Tomer Singer, Martha Noel, Jack Y Huang, Nikica Zaninovic, Owen Davis, Helen Liu, Zev Rosenwaks. *The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA.*
OBJECTIVE : Anti Mullerian hormone (AMH) is a well established biomarker of ovarian reserve. Our objective was to determine if a correlation exists between AMH serum levels and morphological features of transferred embryos in IVF cycles.
DESIGN: Retrospective study
SETTING: Academic medical center-based IVF practice
METHODS: The study cohort was comprised of all patients with serum AMH measurements who underwent IVF treatment in our institution from 01/2008 to 2/2011. Serum AMH samples were drawn prior to initiating IVF treatment and the subsequent completed IVF cycle was used for analysis. The primary outcome was embryo quality, as measured by average fragmentation, grade of top quality embryo, and percentage of 8-cell embryos transferred on day 3 post retrieval . Secondary outcome measures were number of oocytes retrieved , mature and fertilized, number of embryos transferred, number of frozen embryos, antral follicle count, and clinical pregnancy rate.
RESULTS: 190 patients with a total of 490 embryos met inclusion criteria for the study. Patients were subgrouped into those with low AMH (<1.0 ng/mL) and high AMH (>1.0 ng/mL). AMH was found to be positively associated with better embryo quality, as measured by the percentage of 8-cell embryos transferred. AMH was not associated with average percent fragmentation or embryo grade. AMH was also found to be associated with antral follicle count, number of oocytes retrieved, mature oocytes, fertilized oocytes, and number of oocytes frozen. There was no increase in clinical pregnancy rates in patients with higher AMH.
CONCLUSIONS: In our study, AMH levels were significantly associated with measures of higher embryo quality as well as with standard markers of ovarian reserve.

	AMH <1.0	AMH >1.0	p-value
N	109	80	
Age	37.9	36.7	0.056
BMI	23.2	23.1	NS
AMH	0.51	3.04	<0.0001
Antral follicle count[a]	7.25	11.45	<0.0001
Retrieved oocytes[a]	7.67	14.09	<0.0001
Mature oocytes[a]	6.19	11.53	<0.0001
Fertilized oocytes[a]	4.66	8.79	<0.0001
Transferred oocytes[a]	2.57	2.60	NS
Frozen embryos[a]	0.28	1.19	<0.0001
Percentage of 8-cell embryos[a]	41%	55%	0.02
Clinical pregnancy rate (%)[a]	26.1	26.9	NS
[a] Age corrected			

F-208

Embryo Morphology and IVF Outcome in IVF Cycles Using GnRh Agonist vs GnRh Antagonist. Martha Noel, Tomer Singer, Jack Huang, Pak Chung, Nikica Zaninovic, Owen Davis, Zev Rosenwaks. *The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA.*
OBJECTIVE : To determine if the use of GnRh agonist as compared with GnRh antagonist affects embryo morphology and IVF outcome.
DESIGN: Retrospective study
SETTING: Academic medical center-based IVF practice
METHODS: The study cohort was comprised of 153 patients (393 embryos) who underwent IVF treatment in our institution from 01/2008 to 4/2011. IVF cycles were conducted using either GnRh agonist down regulation or GnRh antagonist for endogenous hormone suppression. 37 patients undergoing stimulation with clomid or GnRh agonist co-flare protocol were excluded from the analysis. The primary outcome was embryo morphology as measured by the percentage of 8-cell embryos transferred on day 3 post-retrieval. Secondary outcome measures were IVF cycle outcome including oocytes retrieved, oocytes matured, fertilized oocytes, number of embryos transferred, number of embryos frozen and clinical pregnancy rate. Age, BMI, AMH, AFC, cycle length, total gonadotropin use, and number of previous IVF cycles were also recorded.
RESULTS: There was no difference in embryo morphology in patients undergoing Lleuprolide acetate suppression versus ganirelix suppression. A significant increase was seen in number of embryos frozen in patients receiving GnRh agonist. A significantly higher dose of gonadotropins was administered in patients receiving GnRh antagonist, despite similar AMH levels. No significant difference was seen in retrieved, mature, and fertilized oocytes, transferred embryo number or clinical pregnancy rate between the two groups.
CONCLUSIONS: There was no difference in embryo morphology or IVF cycle outcome between patients stimulated for IVF using Leuprolide acetate as compared to ganirelix.

	Leuprolide acetate	Ganirelix	P-Value
N	52	101	
Age (yrs)	36.5 (±3.82)	37.5 (±4.14)	0.14
BMI (kg/m2)	22.9 (±3.85)	23.1 (±4.23)	0.92
AMH (ng/mL)	1.46 (±2.83)	1.78 (±1.47)	0.44
Antral follicle count	10.5 (±4.58)	8.75 (±4.74)	0.027*
Retrieved oocytes	11.5 (±5.76)	10.5 (±6.09)	0.33
Mature oocytes	9.29 (±4.74)	8.56 (±4.82)	0.37
Fertilized oocytes	7.17 (±4.16)	6.48 (±4.21)	0.32
Transferred oocytes	2.40 (±1.12)	2.65 (±1.07)	0.18
Frozen embryos	1.25 (±1.45)	0.51 (±1.87)	0.007*
8-cell embryos (%)	52.6 (±38.9)	45.2 (±41.3)	0.28
Cycle length (days)	11.0 (±1.98)	10.5 (±1.76)	0.12
Total gonadotropins given	2745 (±1727)	3478 (±1507)	0.008*
Clinical pregnancy rate (%)	24.6	25.0	1.0

F-209

The Ovarian Response to Ovarian Stimulation Treatment Is Beneficially Modified by a Dietary Pattern High in Fruit and Whole Grains. J Twigt,[1] M Vujkovic,[1] J de Vries,[2] J Lindemans,[1] J Laven,[1] R Steegers-Theunissen.[1] *[1]Obstetrics and Gynecology, Erasmus MC; [2]Division of Human Nutrition, Wageningen University.*
Background: The ovarian response to ovarian stimulation treatment is difficult to predict and associates with treatment and reproductive outcome. Side-effects are often due to the high estradiol response. Recently, we demonstrated that preconception folic acid supplement use beneficially lowered the estradiol response to ovarian stimulation treatment. In the current study we investigate whether a specific dietary pattern has a similar effect.
Methods: In 220 subfertile women undergoing ovarian stimulation treatment we assessed nutrient intakes using a Food Frequency Questionnaire and performed principal component factor analysis to identify dietary patterns. The factor most associated with estradiol response was stratified into tertiles of low, medium and high adherence. Blood samples were taken during the early follicular phase of the cycle preceding the treatment cycle and on the day of hCG administration for the determination of estradiol and folate levels. The relation between the identified dietary pattern and the ovarian response was assessed using log-linear linear regression analysis including higher order variables.
Results: A dietary pattern characterized by high intake of fruits, whole grains and margarine and low intake of refined grains and snacks associates with the estradiol response (-0.16, $p<0.05$), baseline serum and red blood cell folate levels (+0.15 and +0.27, $p<0.05$), and BMI (-0.15, $p<0.05$). After adjusting for age, BMI, stimulation protocol and the number of follicles, a one unit increase in adherence to this dietary pattern lowered the estradiol response to stimulation treatment by 14.4% (-24.8% - -3.9%; $p<0.01$). The median number of retrieved oocytes was comparable between the different categories of adherence (low: 7 [4 - 10], medium: 7 [5 - 12], high: 6 [4 - 9]; $p=0.24$). The proportion of pregnancies was considerably higher in the category of highest adherence vs. the two lower tertiles (low: 24.4% vs. high: 34.6%; $p=0.09$).
Conclusion: Adherence to a dietary pattern high in fruit, whole grains and margarine and low intake of refined grains and snacks seems to beneficially modify the endocrine response and the chance of pregnancy after ovarian stimulation treatment. This data support the importance of a healthy preconception diet and confirms the attenuation of the ovarian response also by food folate.

F-210

Markers of Cellular Senescence Are Higher in *In Vitro* Cultured Embryos Compared to *In Vivo* Embryos. Alexandra Meuter,[1] Boris JN Winterhoff,[1] Tamar Tchkonia,[2] Jolene Fredrickson,[1] Theodore Trejo,[1] Lisa M Rogmann,[1] James L Kirkland,[2] Dean E Morbeck.[1] [1]*OB GYN, Mayo Clinic, Rochester, MN, USA;* [2]*Aging Center, Mayo Clinic, Rochester, MN, USA.*

OBJECTIVE: Cellular senescence is characterized by stable cell cycle arrest triggered by a variety of stresses. Senescent cells are viable, metabolically active, and acquire a pro-inflammatory state. The aim of this study was to test if *in vitro* embryo culture induces cellular senescence.

MATERIALS AND METHODS: Single cell mouse embryos were collected from superovulated FVB mice and cultured *in vitro* to the blastocyst stage. *In vivo* developed mouse blastocysts were collected from superovulated FVB mice 4 days after fertilization. *In vitro* and *in vivo* blastocysts were fixed and stained for *senescence-associated-beta-galactosidase (SA-beta-gal)* and *phosphorylated H2A.X (γ-H2A.X)* as well as DAPI. Blastocysts were considered to be SA-beta-gal positive when blue color was evident. Nuclear γ-H2A.X foci were assessed by confocal microscopy and considered positive when more than 4 γ-H2A.X foci were detected. p21, p16, and Interleukin 6 (IL6) were assayed by qRT-PCR.

RESULTS: SA-beta-gal was positive in 76.7% of *in vitro* blastocysts and 3.3% of *in vivo* blastocysts (P<0.0001). Nuclear γ-H2A.X was positive in 45.2% of cells of *in vitro* blastocysts and 14.2% of cells of *in vivo* blastocysts (P<0.0001). p21 mRNA expression was 22.1 fold higher in the *in vitro* than the *in vivo* group (P<0.05), whereas p16 could not be detected. IL6 mRNA was 1.2 fold higher in the *in vitro* group compared to the *in vivo* group (p=0.99).

CONCLUSION: Elevated SA-beta-gal, γ-H2AX, and p21 in cultured embryos compared to *in vivo* embryos suggest that *in vitro* stress can induce a senescence-like phenotype. Markers of cellular senescence were also present in a minority of *in vivo* embryos suggesting that stress occurs at the molecular level *in vivo* and could be environmental or genetic in origin. Clinically, markers of cellular senescence could be used to enhance culture conditions for embryos, as well as a tool for embryo selection.

F-211

Nitrosative Stress Affects Oocyte Quality by Affecting the Metaphase-II Microtubule and Chromosomal Alignment In Vitro. Jashoman Banerjee, Dhiman Maitra, Michael P Diamond, Husam M Abu-Soud. *Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

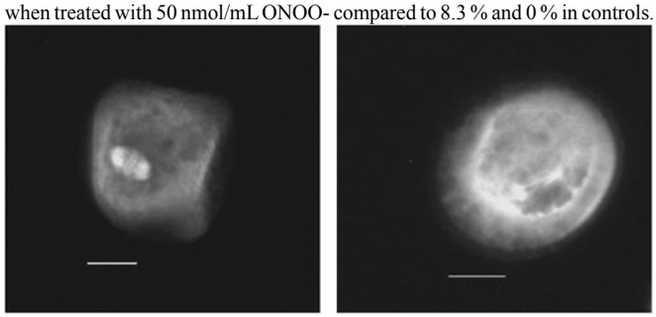
Objective: To investigate the effects of peroxynitrite ($ONOO^-$) on metaphase-II mouse oocyte spindle and chromosomal structure.

Introduction: $ONOO^-$ is a potent oxidant that is generated by the diffusion controlled reaction between superoxide ($O_2^{\bullet-}$) and nitric oxide (NO). Patients with pelvic inflammation and endometriosis have increased $O_2^{\bullet-}$ and NO, which can generate $ONOO^-$. $ONOO^-$ is capable of post-translational modifications of target proteins, including halogenation, nitration, and oxidative cross-linking. We wanted to test the effect of $ONOO^-$on chromosomal alignment and disruption of spindle apparatus of metaphase-II oocyte.

Materials and methods: Metaphase-II mouse oocytes were obtained commercially and incubated in human tubular fluid media at 37° C with 5% CO2 for 60 minutes and then treated for 30 minutes with increasing concentrations of $ONOO^-$ (0-50 nmol/ ml). Oocytes were fixed and stained by indirect immunofluorescence technique to identify the spindle and chromosomal alignment and scored by two different observers based on the table below. Pearson's Chi-square and Fisher's exact test were performed to compare the effects between control and treatment groups.

Score	Microtubule	Chromosome
1		
2		
3		
4	Missing	

Results: 100 % of the oocytes had poor microtubule and chromosome scores when treated with 50 nmol/mL ONOO- compared to 8.3 % and 0 % in controls.



Control **$ONOO^-$ (50 nmol/mL)**

Conclusions: These results show that ONOO- disrupts the metaphase-II mouse oocyte chromosomal alignment and spindle apparatus which could be the underlying cause for poor oocyte quality in patients with endometriosis and pelvic inflammation.

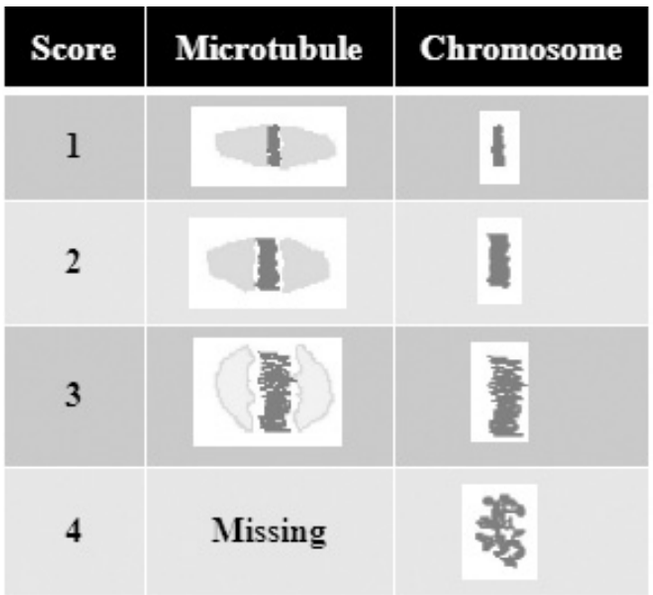
F-212

Melatonin Prevents Hypochlorous Acid Induced Alteration of the Metaphase-II Mouse Oocyte Microtubule and Chromosomal Structure. Jashoman Banerjee, Dhiman Maitra, Faten Shaeb, Ghassan M Saed, Michael P Diamond, Husam M Abu-Soud. *Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

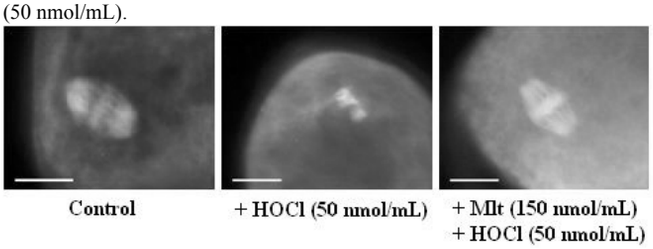
Objective: To investigate the effect of various concentrations of hypochlorous acid (HOCl) on oocyte spindle apparatus disruption and its prevention by melatonin.

Introduction: Patients with endometriosis or pelvic inflammation have poor fertility outcomes. They have higher myeloperoxidase (MPO) and hypochlorous acid (HOCl) levels in the peritoneal fluid. Melatonin, a neurohormone, has been shown to inhibit MPO and scavenge HOCl.

Materials and methods: Metaphase-II mouse oocytes (n=200) were obtained commercially and incubated in human tubular fluid at 37°C with 5% CO_2 for 60 minutes. Oocytes were grouped as: control, melatonin (150, 200 nmol/mL), HOCl (10, 20, 50, and 100 nmol/mL) and HOCl (50 nmol/mL) pretreated with 150 and 200 nmol/mL of melatonin. Oocytes underwent indirect immunofluorescent staining and fluorescent & confocal microscopy to score the alterations.



Results: Pearson Chi-square test, Fisher's Exact tests were used compare outcomes between controls and treated groups and amongst each group. Scores 1 & 2 were combined as good and 3 & 4 as poor outcomes. Poor scores for the spindle and chromosomes increased significantly at 50 nmol/mL of HOCl ($p<0.001$) whereas exposure to melatonin only at 150 and 200 nmol/mL showed no changes. Significant improvement ($p<0.001$) was observed when oocytes were pretreated with melatonin (150 nmol/mL) before adding HOCl (50 nmol/mL).



Conclusions:
1. HOCl alters the metaphase-II mouse oocyte spindle and chromosomal structure in a dose dependant manner.
2. Melatonin prevents HOCl-mediated spindle and chromosomal damage.
3. Melatonin supplementation could be an attractive therapeutic option to improve oocyte quality in endometriosis or inflammatory diseases.

F-213

Expression of Serum Amyloid A in Human Ovarian Follicles: Possible Implication for a Role in Follicular Development. Ronit Haimov-Kochman,[1] Zvesdana Finci-Yeheskel,[2] Ido Eldar,[1] Reinhold P Linke,[3] Mark Levin,[2] Diana Prus,[4] Simcha Urieli-Shoval.[2] *[1]IVF Unit, Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [2]Hematology Unit, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [3]Reference Center of Amyloid Diseases, amYmed, Martinsried, Germany; [4]Pathology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*
Background Serum amyloid A (SAA) is an acute phase protein, expressed primarily in the liver, as a part of the systemic response to various injuries and inflammatory stimuli. SAA is recognized as a modulator of inflammation.
Objective The inflammatory process is key to several ovarian reproductive functions thus**,** studying SAA in the context of ovarian physiology is of importance.
Design, Setting, and Participants Here we investigated the expression of SAA in human ovarian follicles and its presence in follicular fluids, using immunohistochemistry and non-radioactive in situ hybridization analyses applied on ovarian paraffin tissue sections.
Results SAA protein and mRNA expression were detected in follicular cells at all stages of follicular development, from primordial and primary follicles through antral follicles and corpora lutea. SAA was observed in granulosa, theca and luteal cells as well as in oocytes. ELISA analysis detected the SAA protein in follicular fluids of patients undergoing in vitro fertilization (IVF). Levels of SAA in the follicular fluid were somewhat lower than in peripheral blood, yet a strong correlation was found between the two compartments. The SAA protein was also detected in granulosa cells recovered from follicular aspirates of IVF patients and RT-PCR analysis confirmed the trascription of the SAA genes of these cells.
Conclusions These findings indicate for the first time local production of SAA in ovarian follicles, suggesting a role in follicular development.

F-214

Altered Ovarian Perilipin and Adipophilin Expression: A Potential Mechanism for Reproductive Impairment in the Diet-Induced Obese Mouse. Isiah D Harris,[1] William D Schlaff,[1] David Orlicky,[2] Elise Bales,[2] Rhea Sanchez,[2] Brandi Chong,[2] James McManaman.[2] *[1]Obstetrics and Gynecology, University of Colorado, Denver, Aurora, CO, USA; [2]Reproductive Sciences, University of Colorado, Denver, Aurora, CO, USA.*
Objective: To assess the diet-induced obese mouse as a model for obesity related subfertility, and assess ovarian expression of perilipin and adipophilin (ADPH) with obesity.
Materials and Methods: 6 week female C57/B6J mice were fed either a chow diet with 12% calories from fat, or an isocaloric diet with 46% calories from fat. Serum leptin levels were drawn after one week of treatment diet. After 8 weeks on diet, all mice were mated to chow fed, 10 week males. Mice continued their assigned diet through lactation. Mice were then sacrificed and immunohistochemistry was performed on paraffin embedded fresh ovarian tissue.
Results: 35 mice were studied and 9 were fed chow diet. Of the 26 fed a high fat diet, 6 became obese, defined as a final weight greater than 25g and a body fat greater than 20% calculated with MRI (see Table 1). Perilipin and ADPH expression were lowest in the chow fed, higher in the high fat obese, and highest in the high fat lean mice (Table 2).

Clinical Outcomes Stratified by Diet and Obesity

	Chow Fed	High Fat Lean	High Fat Obese	p-value
Weight (g)	22.6	23.7	30.7	>0.01
% Body Fat	12.3%	13.9%	27.4%	>0.01
% Pregnant	78%	45%	50%	0.14
Days to Conception	4.4	6.1	8.0	0.3
# of Pups Per Litter	5.4	7.1	8.3	0.01
Leptin Levels (pg/ml)*	631	922	1700	>0.01

* Leptin levels shown were drawn after one week on the treatment diet

IHC Results

PAT Protein	Cell Type	Tissue/Follicle Type	High Fat Lean	High Fat Obese	p-value
Perilipin	Theca Lutein	Stroma	181%	139%	0.14
		Primary	171%	215%	0.12
		Secondary	160%	110%	0.06
		Antral	152%	115%	0.05
ADPH	Theca Lutein	Stroma	146%	117%	0.21
		Primary	173%	166%	0.05
		Secondary	172%	163%	0.03
		Antral	148%	131%	0.05
ADPH	Granulosa	Primary	127%	79%	0.06
		Secondary	176%	138%	0.11
		Antral	128%	123%	0.16
		Corpra Lutei	150%	152%	0.23

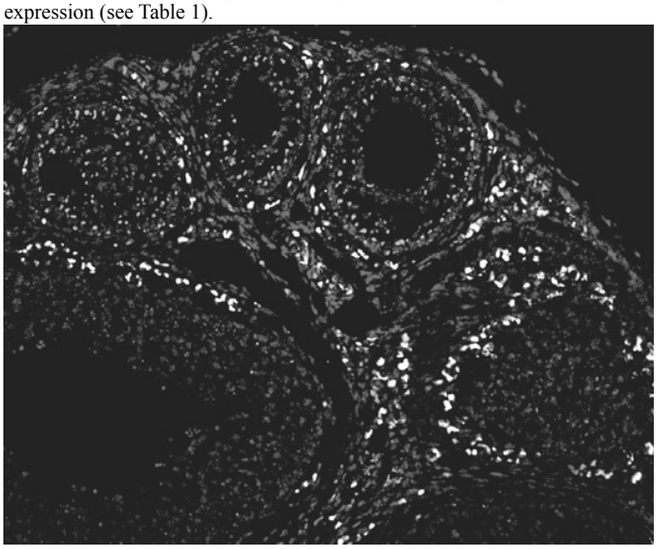
Percentages shown are as compared to Chow Fed mice for each respective cell and follicle type

Discussion: Although diet had a modest effect, obesity was the strongest predictor of reproductive outcomes. The diet-induced obese mouse is an efficient model of obesity related infertility. Increased perilipin and ADPH expression appear to be protective in the high fat lean mice, and the inability to upregulate these proteins may be in the causal pathway between diet and obesity related infertility.

F-215

Characterization of PAT Protein Expression in the Ovary and Ovarian Follicles. Isiah D Harris, David Orlicky, Elise Bales, Brandi Chong, Rhea Sanchez, James McManaman. *Basic Reproductive Sciences, University of Colorado, Denver, Aurora, CO, USA.*
Objective: To characterize PAT protein expression in the ovary and developing follicles.
Materials and Methods: 6 week old, chow fed, female, C57/B6J mice were placed into estrus. 8 mice were studied and 2 mice were randomly assigned to each of the four estrus cycle phases; estrus, proestrus, diestrus A, and diestrus B. Estrus phase was determined by vaginal washes. When a mouse was in an estrus phase consistant with its random assignment, it was sacrificed and immunohistochemistry was performed on paraffin embedded fresh ovarian tissue.

Results: Perilipin was expressed on theca lutein cells and in the stroma, while ADPH was expressed predominately in the stroma and granulosa cells, though also moderately in the theca lutein cells (See Figure 1). TIP47, S3-12 and OXPAT were not expressed in the ovary. Perilipin expression in theca lutein cells was less during the estrus phase in secondary and antral follicles, and ADPH expression was increased in corpra lutei during estrus and proestrus. Otherwise there was no significant interphase variation in perilipin or ADPH expression (see Table 1).



Immunihistochemistry Mean Intensity Scores

	Cell Type	Follicle Stage	Estrus	Proestrus	Diestrus A	Diestrus B	p-value
Perilipin							
	Theca Lutein						
		Stroma	37.1	38.2	39.3	38.4	NS
		Primary	38.4	36.8	54.6	44.1	NS
		Secondary	24.5	48.7	39.9	41.2	0.06
		Antral	21.0	39.8	37.5	52.7	0.01
ADPH							
	Theca Lutein						
		Stroma	15.4	17.9	18.1	16.8	NS
		Primary	10.8	9.9	9.7	7.7	NS
		Secondary	9.5	9.4	9.1	8.3	NS
		Antral	8.9	9.2	8.9	7.9	NS
	Granulosa						
		Primary	15.7	18.4	19.9	9.0	NS
		Secondary	9.1	11.1	9.9	10.2	NS
		Antral	10.2	10.4	9.5	9.8	NS
		Corpra Lutei	21.1	15.3	10.0	8.6	0.05

Mean intensity scores calculated using slidebook 4.2 digital microscopy software. NS = Not significant.

Discussion: This is the first paper to localize the expression of PAT proteins in the ovary. This localization provides insight into the role of PAT proteins in ovarian and follicular physiology, suggesting they are involved in steroidogenesis. Given their function in lipid storage, perilipin and ADPH may be involved in the development of obesity related infertility.

F-216

Luteal Function in Vervet Monkeys (Chlorocebus aethiops). Mila C Kundu,[1] Margaret C May,[1] Justin Chosich,[2] Andrew P Bradford,[2] Nanette Santoro,[2] Susan E Appt,[1] Alex J Polotsky.[2] *[1]Primate Center, Wake Forest University; [2]Ob/Gyn, University of Colorado.*

BACKGROUND: Obesity-related subfertility is associated with reduced production of luteal progesterone. Animal models for this entity are lacking. Vervet monkeys are used to study reproduction,yet corpus luteum function in this species has not been assessed.

OBJECTIVE: To quantify luteal function in vervet monkeys by menstrual cycle excretion of pregnanediol glucuronide (Pdg) and estrone conjugates (E1c).

METHODS: 12 adult female vervets were vaginally swabbed daily to document menstrual bleeding. Urine was collected every 2-3 days for 10 weeks from single-caged monkeys into a clean metal pan placed under the cage. After 4 hours, 5mL of urine was collected. Pdg and E1c were run in duplicate by ELISA and normalized to creatinine. Luteal function algorithm: (1) Pdg nadir during the first 5 samples after menses was noted; (2) a sustained 3-fold rise from the nadir determined evidence of luteal activity (ELA); (3) the day of a 60% drop in the E1c/Pdg ratio in proximity to the Pdg peak defined the day of luteal transition.

RESULTS: 9 monkeys exhibited ELA. Menses ensued 7.7 days on average after the Pdg peak.

Figure 1. Representative hormone pattern in an ELA monkey

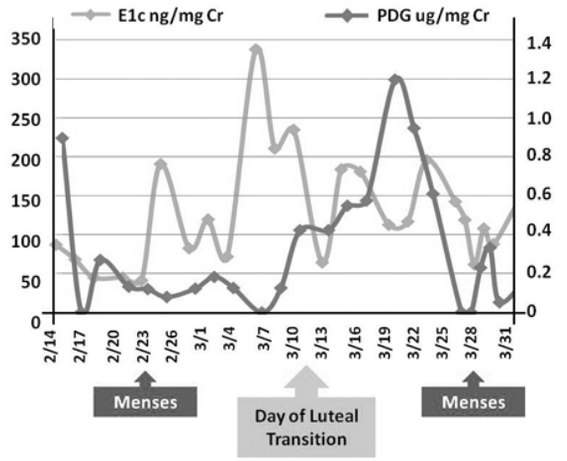
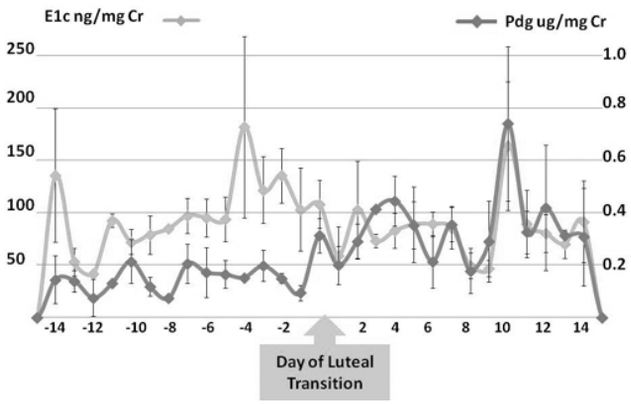


Figure 2. Composite whole cycle hormones for all ovulatory monkeys



Menstrual Cycle Characteristics

	Anovulatory(no ELA)	Ovulatory(ELA)	p
Age, yrs	9.0(2.3)	6.8(2.2)	0.41
Body weight, kg	4.3(0.2)	5.0(0.6)	0.10
% Body fat by DXA	13(3)	17(4)	0.12
Menstrual cycle length, days	32.7(9.3)	29.1(3.0)	0.30
Luteal cycle length, days		12.7(3.3)	n/a

Mean (SD)

All 3 anovulatory monkeys had a rise in E1c (12-fold from nadir on average) followed by a drop that coincided with bleeding.

CONCLUSIONS: Menses could be timed by the preceding Pdg peak. Rise in E1c followed by a drop suggests anovulatory bleeding. Urinary reproductive hormones allow to quantify the corpus luteum function and enable the use of vervets as a model of obesity-related human subfertility.

F-217

Adiponectin Does Not Alter Gene Expression of Anti-Mullerian Hormone and Its Receptor in Human Luteinized Granulosa Cells. Zaher O Merhi,[1] Erkan Buyuk,[1] Davelene Israel,[2] Dara Berger,[3] Athena Zapantis,[3] Streamson Chua, Jr,[2] Sangita Jindal.[3] *[1]Department of Obstetrics & Gynecology and Women's Health, Division of Reproductive Endocrinology and Infertility, Albert Einstein College of Medicine, Bronx, NY, USA; [2]Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA; [3]Montefiore's Institute for Reproductive Medicine and Health, Montefiore Medical Center, Hartsdale, NY, USA.*

Background: Ovarian reserve, reflected by serum anti-Mullerian hormone (AMH) levels, declines with obesity. Obesity is associated with ↑ serum and follicular fluid (FF) leptin (L) and with ↓ serum and FF adiponectin (A).

Friday

We have recently shown that L/A ratio inversely correlates with AMH gene expression in human luteinized granulosa cells (GCs) and that L treatment in culture suppresses gene expression of both AMH and its receptor (R) in GCs. Here we evaluate the effect of A treatment *in vitro* on the expression of these genes in cultured human luteinized GCs.

Materials and Methods: Three women with normal ovarian reserve (mean age= 32 ± 3 years, mean BMI= 24 ± 4 kg/m^2) who underwent controlled ovarian hyperstimulation followed by oocyte retrieval were enrolled. Cumulus and mural GCs from ovarian follicles were collected and pooled separately. Primary GCs of all follicles were split and cultured with either medium alone (DMEM-F12+ 10%FBS) or with medium + A (5 ug/ml) for 24 hrs. Relative gene expression for AMH and AMH-R was measured following culture, with GAPDH as a reference gene. The mRNA was isolated, reverse transcribed and gene expression of AMH and AMH-R was quantified using RT-PCR. Paired-sample Wilcoxon signed rank test was used to assess differences between groups. P<0.05 was considered statistically significant.

Results: Adiponectin treatment in culture did not alter AMH or AMH-R gene expression in cumulus nor in mural GCs.

Gene	GCs	N	Relative Gene Expression (Medium only)	Relative Gene Expression (Medium + A)	Fold Change Gene Expression	p-value
AMH	Cumulus	3	14.1 (9.4-119)	13.4 (9-190)	1.04 (0.6-1.05)	NS
	Mural	2	2.3 (1.4-3.3)	1.9 (1-2.8)	1.3 (1.1-1.4)	
AMH-R	Cumulus	3	3.9 (1.6-17.7)	3.1 (1-36.7)	1.2 (0.5-1.6)	NS
	Mural	2	2.7 (1.5-4)	3.1 (1.5-4.8)	0.9 (0.8-1)	

Data are expressed as median (range). NS= non-significant

Conclusion: These preliminary results indicate that, unlike leptin, adiponectin does not seem to affect AMH or AMH-R gene expression in cultured human granulosa cells.

F-218

Frameshift Single Nucleotide Polymorphism in *BRSK1* Is Not Associated with Premature Ovarian Failure in a Cohort of 60 Patients. Erika M Munch,[1] Alfred Balasa,[1] William E Gibbons,[1] Joe Leigh Simpson,[2] Ertug Kovanci.[1] *[1]Ob&Gyn, Baylor College of Medicine, Houston, TX, USA; [2]Ob&Gyn, Florida International University, Miami, FL, USA.*

Introduction: Genome-wide association studies have identified genetic loci that may be involved in the timing of women's reproductive lifespans. Specifically, these studies have suggested that the *BRSK1* gene may be associated with menopause onset. A single nucleotide polymorphism (SNP rs35068852), located in exon 7 of *BRSK1,* is a G/- frameshift mutation which leads to a premature stop codon. We hypothesized that this frameshift mutation causes a truncation of the protein; therefore, it may be overrepresented in premature ovarian failure patients compared to controls.

Methods: We collected genomic DNA from women with premature ovarian failure (defined as amenorrhea prior to age 40 with elevated FSH >20IU/L) and control patients of similar demographics. A primer set specific for the region of BRSK1 containing exons 5, 6, and 7 was used for polymerase chain reaction to amplify this segment of interest for each sample. The 483-bp PCR product was then sequenced and systematically compared to its Ensembl sequence using BLAST and manually examined chromatograms to identify polymorphisms.

Results: Of 60 patients with premature ovarian failure and 64 controls, we did not detect the G/- frameshift mutation in any sample. No other novel SNPs were identified in exons 5, 6 or 7.

Conclusions: All 3 exons sequenced appear to be highly conserved in women with and without premature ovarian failure, suggesting that BRSK1 mutations are unlikely to cause premature ovarian failure in our population.

F-219

Evidence for Regular Episodic Secretion of Inhibin B, but Not Anti-Müllerian Hormone (AMH), in the Follicular Phase of the Normal Menstrual Cycle Prior to a Mid-Cycle Surge. John F Randolph,[1,2] Margaret E Helmuth,[2] Sioban D Harlow,[2] Daniel S McConnell.[2] *[1]Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA; [2]Epidemiology, University of Michigan, Ann Arbor, MI, USA.*

Objective: Describe variation in inhibin B and AMH in relation to the luteinizing hormone (LH) surge across normal menstrual cycles in reproductive aged women.

Design: Cohort study.

Setting: Academic environment.

Subjects: Twenty regularly-menstruating 30 to 40 year old women.

Interventions: Serum inhibin B, AMH, FSH and LH assayed daily during one normal menstrual cycle.

Main Outcome Measures: Intracycle variability of inhibin B and AMH.

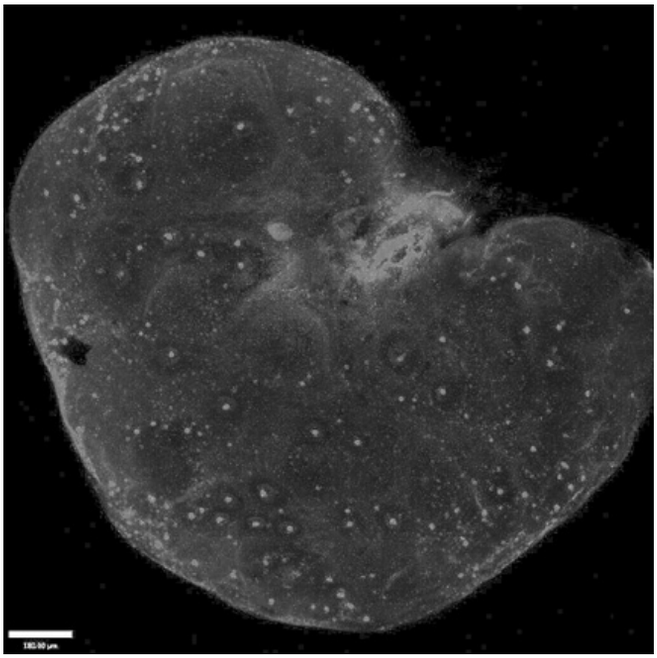
Results: Daily data were aligned to the LH surge. A surge of inhibin B was noted 1.13+0.31 days following the LH surge in 16/20 cycles. Episodic increases in inhibin B of 2-3 days duration occurred in 17/20 cycles 5.29±0.71 days prior to the LH surge, in 14/20 cycles 8.57±0.70 days prior to the LH surge, and in 6/20 cycles 12.00±1.13 days prior to the LH surge. Inhibin B decreased or became undetectable, and did not demonstrate episodic secretion, in the luteal phase. AMH did not demonstrate an episodic secretion pattern. When classified into quartiles of AMH area-under-the-curve (AUC), the lowest AMH AUC quartile 1 levels did not vary across the cycle, whereas AUC quartiles 2-4 showed a progressive mid-follicular increase, mid-cycle decrease, and mid-luteal increase.

Conclusions: Inhibin B secretion in the human menstrual cycle is episodic in the early to mid-follicular phase and immediately after the LH surge, but not in the luteal phase. Anti-Müllerian hormone does not show a similar episodic secretion pattern, but does increase in the mid-follicular phase, decrease at midcycle, and increase slightly in the mid-luteal phase in women in the highest concentration quartiles of AMH secretion. These data suggest different regulatory mechanisms for inhibin B and AMH secretion, and support the concept of 'waves' of follicular development.

F-220

Quantitative 3-Dimensional (3D) Imaging of Whole Mount Adult Mouse Ovary. Amanda Skillern, Svetlana Keylin, Diana Laird. *Department of OBGYN and Reproductive Sciences, University of California, San Francisco, CA, USA.*

Objective: Quantification of primordial follicles (PMFs) is used as an endpoint in both reproductive biology and reproductive toxicology. Traditional methods for quantifying PMF have limitations: variation among observers, tissue sampling bias, and inability to assess qualitative effects on follicles in situ. The objective of this study was to create and optimize a novel strategy to assess ovarian response to stress via PMF counts, morphology of and apoptotic events(AE) in ovarian follicles. Materials and methods: To assess endogenous stress (ovarian aging) and exogenous stress (chemotherapy), ovaries were harvested from mice in these groups: older (8+ months) and younger (6 weeks), mice receiving chemotherapy and those not. Ovaries were fixed, stained with primary antibody(Ab) specific to PMF, granulosa cells(GC), and AE, fluorophore-conjugated secondary Ab, dehydrated, and cleared. Optical sections were collected through the whole ovary with Leica inverted confocal microscope. Stacks were imaged in 3D using Volocity software for quantification of PMF, GC, and AE in follicles. Results: Fixation, penetration, clearing, and Ab binding protocols were optimized. We verified penetration throughout the ovary, visualized Ab signal, and quantified PMFs. We are currently collecting data on quantitative and qualitative differences in these parameters across conditions. Additionally, we are investigating the potential impact of mutant c-Kit pathway on these parameters. Conclusions: Here we present for the first time the use of immunofluorescene in confocal microscopy of whole mount adult mouse ovary. This novel approach allows for mechanization of follicle counting, minimizing interobserver variability. Moreover, by imaging whole ovary, we are able to circumvent sampling bias inherent in ovarian sectioning, as well as assess follicular structures in situ. This model is useful to visualize the effect on chemotherapy on follicular structures when administered in vivo.



F-221

"Hormone of Darkness" and Human Reproductive Processes: Can Melatonin Influence In Vitro Luteal Function? Anna Tropea,[1] Federica Romani,[1] Alessandra Familiari,[1] Elisa Scarinci,[1] Carola Palla,[1] Maria Letizia Uras,[1] Andrea Ciardulli,[1] Stefania Catino,[2] Antonio Lanzone,[2] Rosanna Apa.[1] *[1]Dipartimento per la Tutela della Salute della Donna e della Vita Nascente, Cattedra di Fisiopatologia della Riproduzione, Università Cattolica del S. Cuore, Roma, Italia; [2]Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina (EN), Italia; [3]Roma, Italia.*

Melatonin is an indoleamine involved in the regulation of circadian rhythms, sleep and food-intake. This "hormone of darkness" is primarily synthesized in pineal gland during obscurity and its release is inhibited by light. Apart from the pineal gland, many peripheral tissues express both melatonin and its receptors. This ubiquitous distribution suggests that, in addition to endocrine actions of pineal derived indoleamine, locally produced melatonin might exert autocrine/paracrine effects in different tissues. This should be the case in ovary, where the expression of both melatonin and its receptors has been demonstrated in human granulosa-luteal cells. Actually, previous conflicting data suggest the ability for melatonin to directly modify ovarian function. To further examine the potential direct regulatory role of melatonin in human corpus luteum, in this study we investigated whether melatonin could affect progesterone (P4) release by human luteal cells. Melatonin effect on luteal release of Vascular Endothelial Growth Factor (VEGF), prostaglandin (PG) E2- both luteotropic factors- and luteolytic PGF2α was also evaluated.

Human luteal cells were incubated for 24-48-72h with medium alone (control) or with increasing concentrations of melatonin (10 pM-100 nM) or hCG (100 ng/ml) or CoCl2 (10 mcM), chemical hypoxia. In the culture medium P4, PGs, and VEGF release was assayed by ELISA while VEGF mRNA expression was evaluated by Real-Time RT-PCR.

Our preliminary results demonstrated that melatonin was able to increase P4 and PGE2 release, and to reduce PGF2α. Neither VEGF mRNA expression nor VEGF protein secretion were affected by any tested doses of melatonin. As expected P4 release was significantly increased by hCG, whereas both VEGF mRNA expression and protein secretion by CoCl2.

This study suggests the ability of melatonin to enhance luteal steroidogenesis and to positively influence the balance between luteotropic and luteolytic factors. Our data further support the hypothesis of a direct role for melatonin in regulating luteal function and in keeping corpus luteum integrity.

F-222

Dynamic Changes in Glycosaminoglycan Composition Occur in Term and Preterm Cervical Remodeling. Yucel Akgul, Mala Mahendroo. *OB/GYN and Green Center for Reproductive Biology Sciences, Univeristy of Texas Southwestern Medical Center, Dallas, TX, USA.*

Understanding molecular mechanisms of cervical remodeling is critical for development of therapies and diagnostic tools to prevent premature birth. During pregnancy, extensive changes take place in the cervical extracellular matrix (ECM) that regulates the organization, assembly and tensile strength of collagen fibers. Glycosaminoglycans (GAGs) have diverse functions that regulate macromolecular assembly in the ECM, as well as cell-matrix and cell- cell interactions. These functions are influenced by GAG chain length, sulfation and in the case of proteoglycans, by the protein core to which the GAG is attached. Objective: To quantitatively assess cervical GAGs that is a prerequisite to identify specific GAG functions in cervical remodeling in both term and preterm birth. Methods: GAGs were measured by Fluorophore Assisted Carbohydrate Electrophoresis. Hyaluronan (HA), and sulfated GAGs (sGAGs) that include chondroitin, dermatan, heparin and keratan sulfate were evaluated throughout pregnancy and in preterm birth models. Hyaluronidase activity in human and mouse cervix was determined using fluorescently labeled HA in a FRET assay and through evaluation of HA size. Gene expression was measured by RTQPCR. Results: While sGAG abundance, chain length and sulfation levels were constant through pregnancy, HA increased at term resulting in a net increase in total GAGs and a change in the ratio of HA to sGAGs. Similarly, premature ripening due to infection or progesterone withdrawal was characterized by an increase in HA with no change in sGAGs. The regulation of HA synthesis however, varies between term and preterm models as Has2 gene expression is induced at term while Has1 expression is induced in premature birth models. Similar to studies in mice, there is a transition from high to low molecular weight HA in the human cervix during labor and the timing of these changes correlate with increased hyaluronidase activity or Hyal2 gene expression in mouse and human respectively. Conclusion: These studies identify a shift in sGAG dominance in the nonpregnant and early pregnant cervix to HA dominance in term and preterm cervical ripening. These findings as well as hyaluronidase induced changes in HA size in mice and women suggest important and diverse contributions of HA to macromolecular changes in the ECM that result in loss of tensile strength during parturition and tissue repair postpartum.

F-223

Etiology of Preterm Birth Affects the Degree of Cervical Collagen Reorganization at Birth. Meredith L Akins,[1] Brenda C Timmons,[1] Kate Luby-Phelps,[2] Mala Mahendroo.[1] *[1]OBGYN, UT Southwestern; [2]Cell Biology, UT Southwestern, Dallas, TX, USA.*

OBJECTIVE: Identification of the molecular mechanisms involved in cervical remodeling during term and preterm birth (PTB) is essential for improved diagnostics and therapies to reduce the rate of PTB. Recent studies have identified diverse pathways that drive term ripening, infection-induced and noninfection-induced premature ripening. Because the abundance and organization of collagen is key to cervical function, the focus of this study was to evaluate how collagen remodeling differs in infection and non-infection induced models of PTB.

METHODS: Cervices were collected on gestation day 15 (d15) from mice with premature cervical ripening induced by mifepristone or lipopolysaccharide (LPS) and compared to d15 and d18 controls. Numerous collagen visualization methods were employed to compare collagen organization in premature and term cervical ripening. These included: transmission electron microscopy, collagen I immunofluorescence, second harmonic generation and trichrome staining. We also assessed collagen content in each model. To define the contribution of matrix metalloproteases (MMPs) to collagen disorganization in term and preterm ripening, we evaluated Col1a1 transgenic mice, which are resistant to collagenase cleavage.

RESULTS: Visualization of collagen structure revealed a striking difference in collagen morphology between PTB models. Mifepristone treated cervices appear to have a dramatic disorganization of collagen, which appears more dispersed than term ripening. In contrast, LPS treated cervices show diminished collagen remodeling with minimal changes from the d15 control. Similar to term ripening, the total collagen content and the ratio of fibril collagen I to III remains constant in both PTB models. Further studies will compare the biomechanical properties of the PTB models and the necessity of MMPs to both PTB models will be tested in the protease resistant Col1a1 transgenic mice.

CONCLUSIONS: During normal pregnancy, changes in collagen processing and assembly along with a change in extracellular matrix composition contribute

to collagen disorganization without a loss of total collagen. This study reveals that the process of collagen remodeling in mifepristone and LPS treated cervices differ from normal term pregnancy and from each other. A greater understanding of the divergent mechanisms of cervical ripening in term and PTB will improve diagnostics and therapies.

F-224

Second Harmonic Generation Endomicroscopy: A Potential Clinical Tool for Assessment of Premature Cervical Ripening. Meredith L Akins,[1] Yuying Zhang,[2] Kate Luby-Phelps,[3] Xingde Li,[2] Mala Mahendroo.[1] *[1]OBGYN, UT Southwestern, Dallas, TX, USA; [2]Biomedical Engineering, Johns Hopkins, Baltimore, MD, USA; [3]Cell Biology, UT Southwestern, Dallas, TX, USA.*

OBJECTIVE: Improved diagnostics for the early and accurate assessment of premature cervical remodeling is crucial in order to decrease preterm birth rates. Second harmonic generation (SHG) is an intrinsic property of highly ordered structures, such as collagen. SHG requires no staining, thus it has the potential for imaging live tissue. Recent work has used SHG microscopy in tissue sections to visualize and quantitatively assess progressive changes in murine cervical collagen morphology early in pregnancy. In order to move this technology toward clinical use, an SHG endoscope must be implemented. In this work we describe the validation of a fiber-optic SHG endomicroscopy system that can successfully image and detect morphological changes in cervical collagen fibers throughout gestation.

METHODS: The endomicroscope has an overall 2.0 mm diameter, consisting of a customized double-clad fiber for femtosecond laser delivery and SHG signal collection, a compact PZT actuator for performing high-speed, two-dimensional beam scanning in a spiral pattern, and a miniature compound objective lens of a 0.8 NA for achieving high resolution allowing us to take images with sub-micrometer resolution. Cervices were collected from nonpregnant (NP) and days 6, 12, 15, and 18 of gestation. SHG images of murine cervical tissue sections at different stages of pregnancy were acquired with the endomicroscope and compared to SHG microscopy images. In addition to visual examination, SHG images were also quantitatively analyzed with ImageJ (http://rsbweb.nih.gov/ij/) to extract information on collagen fiber size.

RESULTS: The endoscope system successfully imaged cervical collagen at all time points of gestation. Endoscope images showed previously documented changes in cervical collagen morphology during pregnancy. Assessment of collagen fiber diameter revealed a progressive significant increase from NP to late pregnancy.

CONCLUSIONS: The small form factor of the endomicroscope enables its potential integration with other medical instruments for minimally invasive clinical applications in vivo. Our preliminary results suggest a promising role of SHG endomicroscopy technology for staging normal pregnancy with the potential to assess aberrant cervical remodeling associated with preterm birth.

F-225

Progestins Diminish Cytokine Induced MMP9 Activity in a Human Cytotrophoblast Cell Line Not Expressing the Nuclear Progesterone Receptor. TK Allen,[1] L Feng,[2] C A Grotegut,[2] AP Murtha.[2] *[1]Anesthesiology, Duke University Hospital, Durham, NC, USA; [2]Obstetrics and Gynecology, Duke University Hospital, Durham, NC, USA.*

Introduction: Preterm premature rupture of membranes (PPROM) is associated with global thinning of the chorion layer. Elevated levels of TNFα and proteolytic enymes such as matrix metalloproteinase 9 (MMP9) have been identified in the amniotic fluid of women with PPROM. Recent evidence from our lab has demonstrated that cytokine induced MMP9 activity in fetal membranes can be partially attenuated by progestin analogues. The mechanism involved in this anti-inflammatory effect remains unclear. We hypothesize that this increased MMP9 activity involved in fetal membrane extracellular matrix destruction can be attenuated by progestin analogues and that this mechanism is independent of the nuclear progesterone receptor (nPR). With this in mind, our objective was to evaluate in a human cytotrophoblast cell line (HTR8/SVneo) lacking the nPR, the effect of progestin analogues on cytokine induced MMP9 activity.

Methods: HTR8/SVneo cells were grown to 60% confluency and incubated with ethanol vehicle (control), progesterone (P4), 17α hydroxyprogesterone (17P) and medroxyprogesterone acetate (MPA) at a concentration of 10-6 M for 1 h followed by TNFα (10 ng/ml). Culture media were harvested after 24 h and MMP9 proteolytic activity measured using substrate gel zymography. Densitometric analysis was performed using Image J software (NIH). Results were pooled as mean (±SEM) and treatment groups compared using the Kruskal Wallis test with post hoc pairwise comparisons using the Dunn's multiple comparison test.

Results: TNF α induced a significant increase in MMP9 activity when compared to controls ($p<0.001$). Pretreatment with MPA resulted in a 40% reduction in MMP9 activity when compared with TNF α ($p<0.01$). There were no significant reductions observed with the other treatments.

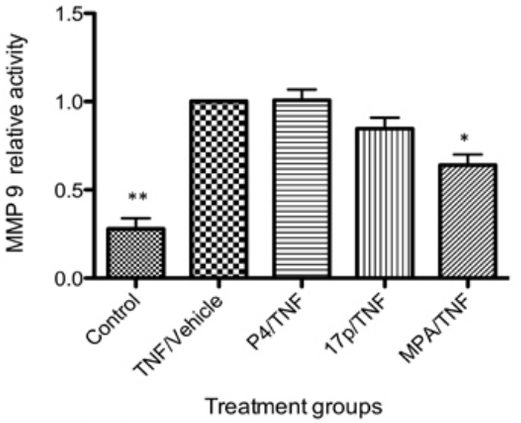


Figure 1. Effect of treatments on MMP 9 activity. MMP 9 activity is relative to the TNF/vehicle group.*P< 0.01, **P<0.001 vs TNF/vehicle group. Data are mean ± SEM from 5 replicates.

Conclusion: These findings suggest that the mechanism involved in the attenuation of MMP9 activity observed with progestins is independent of nPR. This mechanism is being currently investigated in our lab.

F-226

Elective Cervical Cerclage for Late Miscarriages; a Retrospective Observational Study. Holly V Lewis, Mohammad Aziz, Melody Taheri, Nancy Alleyne, Kathyrn Birdsall, Raj Rai, Lesley Regan. *Obstetrics and Gynaecology, St Mary's Hospital, London, United Kingdom.*

Pre-term birth is the single most important determinant of perinatal mortality and morbidity. Late miscarriage is a significant risk factor for subsequent pre-term birth. Elective cervical cerclage can be performed for women with a history suggestive of cervical weakness. Uterine cavity abnormalities and thrombophilic disroders have also been shown to increase the risk of pre-term delivery.

Objective: To examine a) live birth rate and b) gestational age at delivery amongst women who underwent modified shirodkar cervical cerclage.

Methodology: Jan 2004 and March 2010, 129 women attending a tertiary recurrent miscarriage clinic at St Mary's Hospital, London underwent a modified shirodkar cervical cerclage at 12-14 weeks gestation under antibiotic cover. It was performed under GA by one of two senior obstetricians. All pregnancies were singleton. No woman received progesterone.

Results: Median age of these women was 35 years (average 22-44y).

96 women had a coexistent thrombophilic abnormality (antiphospholipid syndrome, abnormal thromboelastogram, genetic thrombophilia mutations) and treated with acombination of aspirin and heparin or aspirin alone. 10 women who were found to have a uterine septum, underwent hysteroscopic resection prior to conception.

Live birth rate was 93% (120/129). There were 8 late miscarriages and 1 intrauterine death at term due to placental abruption.

Among the 120 live births, 114 (95%) delivered after 34 weeks.All 6 women who delivered before 34 weeks had thrombophilic disorders. Three presented with pre-term labour, 2 with PPROM and 1 with severe PET nd IUGR necessitating emergency caesarean section.

Conclusion:

Women presenting with late miscarriage frequently suffer from multiple contributary factors.

Elective cervical suture at 12-14 weeks gestational and treatment of coexisting thrombophilic disorders results in a high live birth rate.

F-227

Cervical Biomechanical Changes in Rat Models of Preterm Labor. Justine Chang,[1] William Barone,[2] Steven Abramowitch,[1,2] Hyagriv Simhan.[1] *[1]Magee-Womens Research Institute, Dept of OBGYN, University of Pittsburgh, Pittsburgh, PA, USA; [2]Dept of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA.*

BACKGROUND

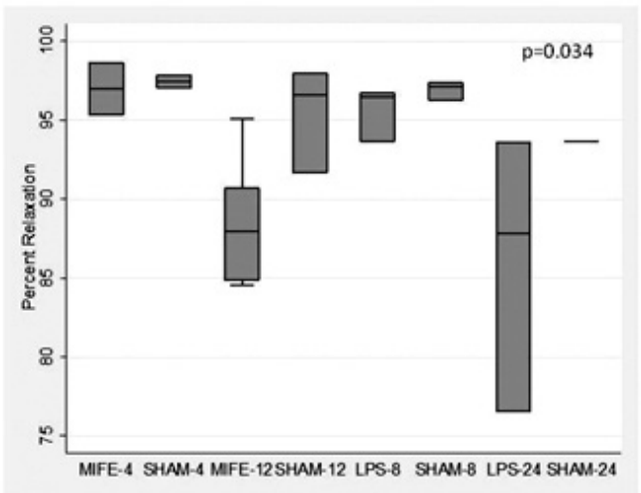
Cervical biomechanical properties change in pregnancy and parturition. We aim to explore changes in viscoelastic behavior in a rat model of preterm labor (PTL) induced by progesterone receptor antagonism or intrauterine inflammation.

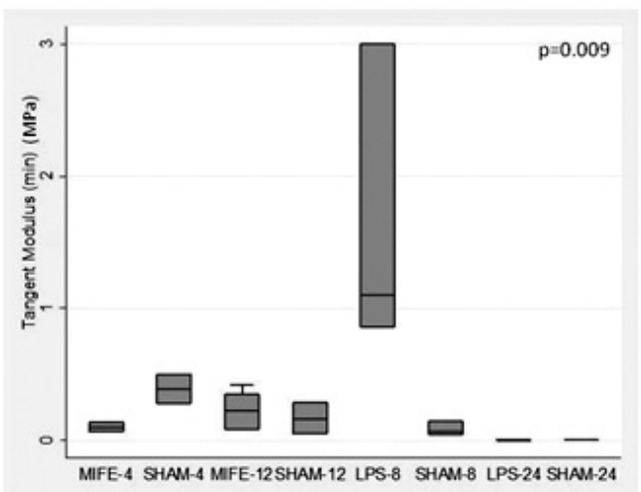
METHODS

Long-Evans timed-pregnant rats had mifepristone (MIFE) injection or intrauterine lipopolysaccharide (LPS) injection on d15. Cervices were obtained at 4 & 12 hrs for MIFE and at 8 & 24 hrs for LPS, corresponding to 16% & 50% of time to delivery (n=1-7); sham controls for each group were collected at the same time points. Cervices were divided into proximal and distal sections. Biomechanical properties were evaluated with unconfined compression testing. Peak stress, percent relaxation, and tangent moduli (minimum and maximum slopes of the stress-strain curve) were determined. Peak stress and percent relaxation change with differences in tissue water content and porosity; tangent modulus reflects tissue stiffness.

RESULTS

As there was a trend towards differences in biomechanical parameters between distal and proximal cervix within groups, all comparisons were stratified by position. Kruskal-Wallis testing showed significant differences between groups in percent relaxation (p=0.034) and minimum tangent moduli (p=0.009). In distal cervix, sham controls had a higher percent relaxation than PTL. In proximal cervix, minimum tangent moduli were highest for LPS-8; LPS-24 had values similar to controls. Although not statistically significant, a similar trend was seen for maximum tangent moduli. Tangent moduli did not change in MIFE. No significant differences were noted in peak stress between groups.





CONCLUSION

PTL tissues may have a lower percent relaxation than control pregnant tissues due to differences in water content and porosity of the solid matrix which lead to changes in rates of dissipation of applied forces. LPS-8 appeared to have a stiffer tissue response than seen in MIFE; this may reflect different cervical biochemical changes resulting from different etiologies of PTL.

F-228

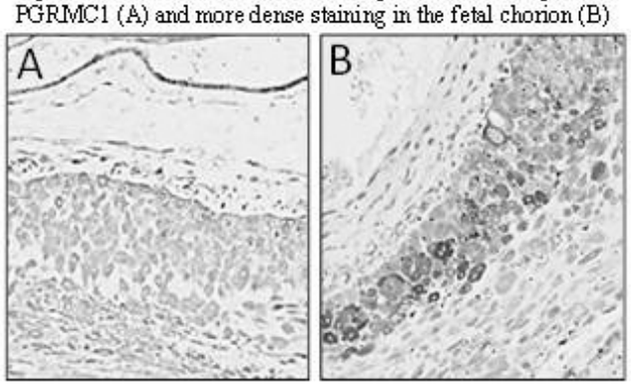
PGRMC1 Expression in Fetal Membrane Layers among Women with Preterm Premature Rupture of the Membranes (PPROM). Liping Feng, Brian Antczak, Jennifer Thompson, Chad A Grotegut, Amy P Murtha. *OB/GYN, Duke University Medical Center, Durham, NC, USA.*

Objective: Prior work demonstrated thinning of the chorion layer of fetal membranes and apoptosis among PPROM subjects. Further, PGRMC1 function has been implicated in mediating cell death in primary cultured chorion cells from fetal membranes. Our objective was to determine if PGRMC1 expression levels in fetal membrane vary by cell layer or clinical status.

Methods: Fetal membrane samples collected from sites distant from rupture in PPROM (n=10), preterm no labor (PTNL, n= 9) and term no labor (TNL, n=10) subjects were formalin fixed and stained using PGRMC1 antibody (Sigma, St. Louis, MO). Images obtained using an Axio Imager to determine location and distribution of PGRMC1. Relative staining of PGRMC1 in each layer of fetal membrane was quantified by a 5 point scoring system. All investigators obtaining, and scoring images were blinded. Data were analyzed by Kruskal Wallis or Mann Whitney (Analyse-It, UK).

Results: PGRMC1 protein was detectable in cytoplasm of cells from all layers of the fetal membrane. The median PGRMC1 staining among all cell types was lower in PPROM subjects compared to PTNL and TNL (3.1 vs. 4.4 vs. 3.4; P=.009). When the chorion layer was analyzed separately, median PGRMC1 expression was lowest in PPROM compared to PTNL and TNL (2.9 vs. 4.4 vs. 3.5, P=.09). Comparisons for decidua and amnion were not significant. When PPROM subjects were excluded, PGRMC1 expression was less in TNL subjects compared to PTNL (4.4 vs. 3.4, P=.03) among all cell types. In some subjects PGRMC1 expression is consistent in all cell layers but in others differences exist between layers of the fetal membranes.

Figure: Fetal membrane demonstrating uniform staining for PGRMC1 (A) and more dense staining in the fetal chorion (B)



Conclusions: These results suggest that all fetal membrane cell types are relevant for PGRMC1-mediated progesterone action, especially cells of fetal origin (amnion and chorion), which lack nuclear progesterone receptor. PGRMC1 expression appears to be actively regulated in fetal membranes and decreased in term subjects. Importantly, in PPROM (at sites distant from rupture), PGRMC1 expression appears to be diminished especially in fetal chorion cells.

F-229

The Role of Vitamin D_3 on hCAP18/LL37 Expression in the Lower Female Genital Tract. Lorraine Frew,[1] Andrew T McKinlay,[1] Rosemary Leask,[1] Donald J Davidson,[2] Jane E Norman,[1] Sarah J Stock.[1] *[1]University of Edinburgh/MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom; [2]University of Edinburgh/MRC Centre for Inflammation, University of Edinburgh, Edinburgh, United Kingdom.*

Background: Antimicrobial peptides (AMPs) are small peptides produced by epithelial surfaces, important in infectious and inflammatory conditions. They may have a role in preventing infection associated with preterm labour. The AMPs hCAP18/LL37 (cathelicidin) and human beta defensin 2 (HBD2) have synergistic antibacterial and immunomodulatory effects which can be regulated by vitamin D_3.

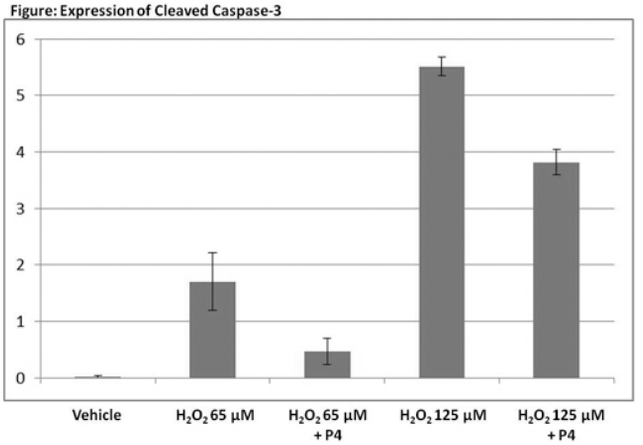
Friday

Aim: To determine whether hCAP18/LL37 and HBD2 are expressed in cervicovaginal secretions and cells derived from the lower genital tract and whether expression is regulated by vitamin D_3.
Methods: Ethical approval was obtained (REC ref S1103). Cervicovaginal secretions were obtained at speculum examination or self collected. Production of hCAP18/LL37 and HBD2 cervicovaginal secretions was determined by enzyme linked immunosorbant assay. Three immortalized cell lines derived from endocervix (END), ectocervix (ECT) and vaginal (VK2) epithelium were cultured *in vitro* with a dose response of active 1, 25-OH and inactive 25-OH vitamin D_3. The expression of hCAP18/LL37 gene *CAMP* and HBD2 gene *DEFB4* was determined by real time qPCR. Data was analysed by one way analysis of variance.
Results: hCAP18/LL37 and HBD2 expression was detected in cervicovaginal secretions, with a median concentration of 15.4ng/ml and 212.7ng/ml respectively (n=3-11). The expression of *CAMP* was up-regulated by treatment with 100nM 1,25-OH vitamin D_3 (n=3, $p<0.01$) and by treatment with 100nM 25-OH vitamin D_3 (n=3, $p<0.001$) compared to untreated conrols. The expression of *DEFB4* was unaffected by vitamin D_3 in al three cell lines.
Conclusion: We have shown that hCAP18/LL37 and HBD2 is a component of cervcovaginal secretions in pregnant women. Cells derived from the lower genital tract express hCAP18/LL37 and this is regulated by vitamin D_3 *in vitro*.

F-230

Progesterone Protects Chorion Cells from Apoptosis Induced by Reactive Oxygen Species. Jennifer B Gilner, Amy Murtha, Liping Feng. *Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA.*
Background: Progesterone is used in clinical practice to prevent recurrent preterm delivery, however the molecular mechanism of its effect is incompletely understood. Oxidative stress plays an important role in apoptotic cell death and has been implicated in preterm premature rupture of membranes (PPROM). We hypothesize that apoptosis resulting from oxidative stress can be inhibited by progesterone and that this mechanism is independent of the nuclear progesterone receptor (nPR). Our objective was therefore to determine, in a human cytotrophoblast cell line (HTR8/SVneo) lacking nPR, the effect of progesterone treatment on oxidative stress-induced apoptosis.
Materials and Methods: HTR8/SV neo cells were pretreated with or without progesterone (P4, 10^{-6} M) for 1 h followed by H_2O_2 (65 μM or 125 μM) treatment for 24 h. Five hours into H_2O_2 treatment, P4 was re-dosed. Western blot was performed after 24h H_2O_2 treatment to assay the amount of cleaved caspase-3 (apoptosis marker) expression induced by H_2O_2 (65 μM or 125 μM). Densitometry was performed by normalizing to GAPDH expression and data analyzed by Kruskal Wallis (Analyze-It, UK).
Results: Prolonged H_2O_2 exposure induces apoptosis (as measured by expression of cleaved caspase-3) in cytotrophoblast cells in a dose-dependent fashion. In cultures pretreated with P4, expression of cleaved caspase-3 is significantly reduced (0.69-fold in 65 μM treatment group and 0.27-fold in 125 μM treatment group; $p=0.05$).

Figure: Expression of Cleaved Caspase-3



Conclusion: Progesterone pretreatment protects chorion cytotrophoblast cells, lacking nPR, from H_2O_2-induced apoptotic cell death. This protective effect is one mechanism by which progesterone may reduce PPROM-related preterm births.

F-231

The Presence of Mitochondrial Progesterone Receptors in the Amniochorionic Membranes. Catherine Herway,[1] Xiaochuan Chen,[2] Miranda Anderson,[2] Kathryn Reed,[1] Sean Limesand.[2] *[1]Obstetrics and Gynecology, University of Arizona, Tucson, AZ, USA; [2]Animal Sciences, University of Arizona, Tucson, AZ, USA.*
Introduction: Preterm Premature Rupture of Membranes (PPROM) is a major cause of neonatal morbidity and mortality and remains poorly understood. Progesterone is recommended for women with a prior history of PPROM as a preventative measure in subsequent pregnancies. However, most women have no prior history of PPROM, and therefore are not offered progesterone supplementation. High concentrations of mitochondrial progesterone receptor (PR-M) mRNA are found in the myometrium of pregnant women. The maternal role for PR-M has not been resolved. If present in the amniochorionic membranes, this may provide a second potential site for PR-M to act as a mediator in premature rupture of membranes in the absence of labor. The objective of this study was to determine whether PR-M was expressed in the amniochorionic membranes.
Methods: Placenta, chorion, and amnion samples were collected from three human subjects. One patient was diagnosed with preterm labor and delivery at 30 3/7 weeks gestation, and the remaining two patients experienced spontaneous vaginal deliveries at 38 2/7 and 39 4/7 weeks. Upon delivery of the placenta, the specimens were immediately placed in a cold Mannitol-based buffer and transported to the laboratory for dissection. A portion of each tissue was snap frozen and stored at -800C until RNA extraction with Tri Reagent. Synthetic oligonucleotide primers designed to unique regions for PR gene (Accession # AY212933.1) were examined by RT-polymerase chain reaction (PCR) to identify PR-A,-B, or -M isoforms. Amplified cDNA was separated on a 1% agarose gel, stained with EtBr and verified by nucleotide sequencing.
Results: PR-M transcripts were found in placentae and chorionic membranes of all patients. PR-M was not detected in the amnionic membranes. Both PR-A and PR-B RNA transcripts were present in all tissues collected. Additional confirmation of PR-M presence in the chorion using the western blot and immunohistochemistry techniques are currently underway.
Discussion: PR-M transcripts are present in the chorionic membranes and could signify their involvement as an intermediary of premature rupture of membranes in the absence of labor. Localization of the PR-M may advance our understanding for progesterone regulation of membrane integrity.

F-232

A Co-Culture Model of the Endocervical Epithelium Using Primary Human Cervical Stromal Cells and Epithelial Cells. Michael D House,[1] David L Kaplan.[2] *[1]Obstetrics and Gynecology, Tufts Medical Center, Boston, MA; [2]Biomedical Engineering, Tufts University.*
Objective: Interactions between the endocervical epithelium and cervical stroma have been implicated in both cervical softening and cervical ripening during pregnancy. Our objective was to develop an in vitro co-culture model of the endocervical epithelium using primary human cervical stromal cells and endocervical epithelial cells.
Methods: Cervical cells were isolated from explants of cervical biopsies from non-pregnant women. Epithelial cells were isolated by culturing explants in media specific keratinocytes (Invitrogen). After the first passage, epithelial cells were culture expanded on a feeder layer of irradiated 3T3 cells (ATCC). Cells from passage 2 - 3 were used for experiments. Stromal cells (fibroblasts) were isolated by culturing explants in DMEM containing 10% FBS. Immunofluorescence with markers specific for epithelial cells (pancytokeratin) and fibroblasts (vimentin) was performed to confirm cell lineage. The co-culture was performed using a collagen gel technique. A collagen solution (1 mg/mL) was neutralized, seeded with fibroblasts (125,000 cells per gel), and brought to room temperature in the transwell of a six well plate. After four days, a contracted collagen gel was seen. A concentrated solution of epithelial cells (250,000 cells/cm^2) was applied to the surface of the gel. A stainless steel ring was used to keep the epithelial cell solution localized. The gels were assessed for viability (LIVE/DEAD reagent), immunohistochemistry (IHC) for pancytokeratin and secretion of IL-6 (ELISA). On control gels, no epithelial cells were applied.
Results: Uniform populations of epithelial cells and fibroblasts were confirmed by morphology and immunofluorescence. The LIVE/DEAD assay and confocal microscopy showed a viable epithelial surface on the fibroblast-populated collagen gel. IHC for pancytokeratin revealed the epithelial layer resembled native cervical tissue. ELISA showed significantly increased baseline secretion of IL-6 when an epithelial layer was present ($p=.01$).

Conclusion: Fibroblast populated collagen gels with an epithelial surface could be useful for studying stromal-epithelial interactions in a controlled in vitro environment, which will improve our understanding of mechanisms responsible for cervical softening and ripening.

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Stretch Modulate ITGA11 Expression and Collagen Production in Rat Cervical Stroma Cells through Activation of JNK and Erk1/2 Pathways. Huiling Ji, Edward K Chien. *Obstetrics and Gynecology, Maternal Fetal Medicine, Warren Alpert Medical School of Brown University, Providence, RI, USA.*

Objective

We recently reported changes in the expression of collagen binding Integrin alpha 11 (ITGA11) in cervical tissue at the end of gestation. The pattern of expression suggested that ITGA11 may be regulated by progesterone. However, studies using progesterone antagonists showed no alterations in expression of ITGA11 to progesterone withdrawal in cervical tissue. We hypothesized that mechanical stretch may be the primary regulator of ITGA11expression in cervical tissue. We evaluated the effects of stretch on rat cervical stromal cells (RCS) and ITGA11 expression and its impact on collagen production.

Study design

Primary RCS cultures were generated from non-pregnant rats using a tissue explants method approved by our local IACUC. RCS were cultured in DMEM with Fetal Bovine Serum (FBS). Passages 2-6 were used for experiments. RCS were plated on Flexcell plates and cultured in DMEM with charcoal treated FBS to remove residual hormones. The effect of 5% cyclical stretch was evaluated and compared to non-stretched controls. Cells and supernatant were harvested at various time points for protein expression and collagen assay. The MAPK inhibitors (SP600125 or U0126) were also evaluated to block MAPK signaling. Soluble collagen was assayed using Sircol Collagen Assay kit.

Results

Stretch decreased the expression of ITGA11 and increased soluble collagen production compared to non-stretched controls. Stretch activated both the JNK and ERK signaling pathways. Increased phosphorylation of JNK and Erk1/2 was observed after 15mins of stretch. Both SP600125, JNK activity inhibitor, and U0126, inhibitor of Erk1/2 phosphorylation, blocked stretch induced the down-regulation of ITGA11 expression and up-regulation of soluble collagen production.

Conclusions

Mechanical stretch decreased the expression of ITGA11 and increased the production of collagen in RCS through activation of JNK and Erk1/2. Down-regulation of ITGA11 may be an important mechanism associated with cervical ripening and decreased mechanical integrity at the end of pregnancy.

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Endocervical Immune Mediator Production Following Successful Ultrasound Indicated Rescue Cerclage Placement. Tomi Kanninen,[1] Catherine Herway,[2] Daniel W Skupski,[2] Ann Marie Bongiovanni,[1] Gary S Eglinton,[2] Steven S Witkin.[1] *[1]Obstetrics and Gynecology, Weill Cornell Medical College, New York City, NY, USA; [2]Obstetrics and Gynecology, New York Hospital Queens, Flushing, NY, USA.*

Introduction Placement of a cervical cerclage at mid-trimester in women at risk for preterm labor is a common procedure with apparent benefits for some women. However, the changes that occur in the cervix following this procedure remain incompletely identified. We evaluated the endocervical concentrations of mediators involved in extracellular matrix (ECM) stabilization or degradation prior to, and up to 120 days following, cerclage placement.

Methods The study population was 53 women who underwent an ultrasound-indicated or a rescue cerclage at 15 to 25 weeks gestation due to a cervical length <1.5 cm. All delivered a healthy neonate at term. Samples were tested by ELISA for concentrations of hyaluronan (HA), 27 kDa heat shock protein (hsp27), transforming growth factor-b (TGF-b), extracellular matrix metalloproteinase inducer (CD147/EMMPRIN), and matrix metalloproteinase (MMP) – 1 and - 8.

Results Concentrations of both HA and hsp27 were highest at the time of cerclage placement and then decreased while TGF-b and EMMPRIN increased in concentration following the procedure. The highest mean EMMPRIN level was measured at >90 days following the procedure while TGF-b levels peaked at 61-90 days post-cerclage. MMP-1 and MMP-8 were not detected over the study time period.

Discussion In women with a successful cerclage placement the selective regulation of mediators inhibits progression of ECM degradation and cervical ripening. Successful outcome with cervical cerclage was associated with increases over time in TGF-b and EMMPRIN and a decrease in the overall concentration of HA and hsp 27. The relationship between TGF-b and EMMPRIN may modify the synthesis of HA or the induction of MMPs decreasing ECM breakdown and thereby decreasing total HA and hsp 27 concentrations. The continued study of factors predictive of success following cerclage placement and cervical quiescence may allow an improved evaluation of prospective patients.

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Identification of Biomarkers Associated with Approaching Spontaneous Preterm Labor in Asymptomatic Women. Stella Liong,[1,2] Megan KW Di Quinzio,[1,2] Michael Permezel,[1,2] Harry M Georgiou.[1,2] *[1]Obstetrics & Gynaecology, University of Melbourne; [2]Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, VIC, Australia.*

Background: Preterm birth is the delivery at <37 weeks' gestation and affects approximately 8-13% of all deliveries worldwide. It is a significant contributor to infant mortality and long-term morbidity, including increased risk of severe impaired neurodevelopment and respiratory complications. Our objective was to investigate the biochemical changes that are reflected in the cervicovaginal fluid (CVF) of asymptomatic women who subsequently experienced spontaneous preterm labor (PTL).

Subject: CVF swabs were collected from asymptomatic women attending Antenatal Clinic who were deemed at risk of delivering preterm. Risk factors for prospective recruitment included previous history of spontaneous preterm delivery or preterm pre-labor rupture of membranes, and multi-fetal gestation. The PTL group consisted of 5 women who spontaneously delivered within 11-22 days. Women with spontaneous term delivery outcomes, recruited from the same population, served as the gestation-matched controls for this study (n=10).

Methods: Two-dimensional difference in gel electrophoresis (2D-DIGE) was employed to identify possible CVF biomarkers of impending preterm labor. Spots of interest were subjected to mass spectrometry (nanoLC-ESI-MS/MS) for identification and validated using Western blot techniques.

Results: A total of 13 unique differentially expressed proteins were present in the CVF of the PTL and control groups. Protein expression that decreased at 11-22 days before PTL were the fatty acid binding protein 5 (2.5-fold); apolipoprotein A1 (2.8-3.7-fold); transaldolase (6.3-fold); serpin B1 (MNEI; 1.8-2.0-fold); serpin B3 (SCCA-1; 1.4-fold); vitamin D binding protein (1.4-3.7-fold); cystatin A (stefin A; 3.2-fold); thioredoxin (2.8-fold); interleukin-1 antagonist receptor (1.4-fold) and Cu,Zn-superoxide dismutase (2.9-fold). In contrast, 3 identified proteins were shown to increase prior to the onset of PTL: serum albumin (1.4-3.6-fold); glutathione-S-transferase-pi (2.4-fold) and serpin B6 (placental thrombin inhibitor; 3.6-fold).

Conclusion: There are diverse biochemical changes that are reflected in the CVF of asymptomatic women 11-22 days before spontaneous PTL. The presence of these differentially expressed proteins with approaching labor may help in the development of a potential diagnostic test and improved management in materno-fetal health.

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Biomarker Discovery in Symptomatic Women with Impending Preterm Labor. Stella Liong,[1,2] Megan KW Di Quinzio,[1,2] Michael Permezel,[1,2] Harry M Georgiou.[1,2] *[1]Obstetrics & Gynaecology, University of Melbourne; [2]Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, VIC, Australia.*

Background: The accurate risk assessment of preterm delivery in women presenting with symptoms of threatened preterm labor (PTL) is limited. Diagnosis is further confounded when symptomatic women do not present with observable cervical change. Therefore, the subsequent treatment and management of these women rely on the outcome of the fetal fibronectin test. This test has limited applications due to its poor positive predictive value and low sensitivity[1] and is routinely used for its high negative predictive ability. The aim of this study was to investigate the global changes within the cervicovaginal fluid (CVF) proteome of symptomatic women with threatened PTL who subsequently progressed to preterm delivery.

Subjects: A CVF swab was collected from women presenting to the Emergency Department with symptoms of threatened PTL (defined as painful uterine contractions without observable cervical dilatation or effacement). Women who subsequently delivered preterm served as the 'true' threatened PTL group (n=4, >40 days from spontaneous PTL). Gestation-matched women presenting with threatened PTL who delivered at term served as the control group (n=8)

Methods: Two-dimensional difference in gel electrophoresis (2D-DIGE) was used to examine the differential protein expression in symptomatic women

who either delivered preterm or at term. Proteins of interest were subjected to mass spectrometry (nanoLC-ESI-MS/MS) for identification and subsequently validated using Western blot analysis.
Results: A total of 14 unique differentially expressed proteins were found in women who delivered preterm. The biological roles of the identified proteins are as follows: metabolism (fatty acid-binding protein 5, ↓2.1-fold; transaldolase, ↓2.9-fold, vitamin D binding protein, ↑7.9-fold), oxidative balance (thioredoxin-1, ↓2.9-fold; peroxiredoxin-2, ↑2.2-fold), immune response (calgranulin B, ↑3.1-fold; interleukin-1 receptor antagonist, ↓1.7-fold) and protease inhibition (serpin B1/MNEI, ↓1.9-fold; serpin B6, ↓1.7-fold).
Conclusion: These results demonstrate distinct differences in the CVF proteome between symptomatic women who subsequently deliver preterm compared to those who deliver at term. The discovery of novel differentially expressed proteins may help improve the assessment of symptomatic women who progress to deliver preterm.
1. Leitich H et al. 1999. Am J Obstet Gynecol, 180:1169-1176

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Biomarkers of Impending Preterm Pre-Labor Rupture of Fetal Membranes. Stella Liong,[1,2] Megan KW Di Quinzio,[1,2] Michael Permezel,[1,2] Harry M Georgiou.[1,2] *[1]Obstetrics & Gynaecology, University of Melbourne; [2]Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, VIC, Australia.*
Background: Preterm pre-labor rupture of membranes (PPROM) is the rupture of the fetal membranes prior to the onset of labour at less than 37 weeks' gestation. PPROM accounts for approximately 30% of preterm births and is associated with chorioamnionitis, neonatal sepsis and placental abruption, leading to high risks of perinatal morbidity and mortality. The objective of this study was to identify differentially expressed proteins in the cervicovaginal fluid (CVF) of asymptomatic women before the clinical manifestation of PPROM.
Subjects: Asymptomatic pregnant women were prospectively recruited for CVF sample collection. The PPROM group consisted of women with samples collected within 6-23 days before rupture of membranes and who subsequently delivered preterm (n=5). Women with normal spontaneous term delivery outcomes served as gestation-matched controls (n=10).
Methods: Two-dimensional difference in gel electrophoresis (2D-DIGE) was used to distinguish differential expression between the pooled cohorts. To examine the biological variation within groups, each individual sample was subjected to 2D-polyacrylamide gel electrophoresis (2D-PAGE) analysis. Spots of interest were identified by mass spectrometry (nanoLC-ESI-MS/MS) and validated using Western blot.
Results: Proteomic analysis of the CVF revealed differing expression profiles between the PPROM and term control groups. Proteins that were significantly decreased in the PPROM cohort included: thioredoxin (2.3-fold; p=0.013); interleukin-1 antagonist receptor (1.6-fold; p=0.005); fatty acid-binding protein 5 (2-fold; p=0.005), cystatin A (stefin A; dimer; 2.6-fold; p=0.028), serpin B1 (MNEI; 1.5-fold; p=0.028) and serpin B3 (SCCA-1; 2.7-fold; p=0.028). In contrast, annexin A3 (3.8-fold; p=0.005) and vitamin D binding protein (2.3-fold; p=0.005) were significantly increased 6-23 days before PPROM. These proteins have known biological functions in oxidative balance, anti-inflammatory activity, metabolism or protease inhibition that may impact on cervical dilatation and weakening of the fetal membranes.
Conclusion: We have identified several proteins that are differentially expressed within 6-23 days before PPROM. The development of a test to assess the risk of an impending PPROM event in clinically asymptomatic women may in the future provide better management and improved maternal and fetal outcomes.

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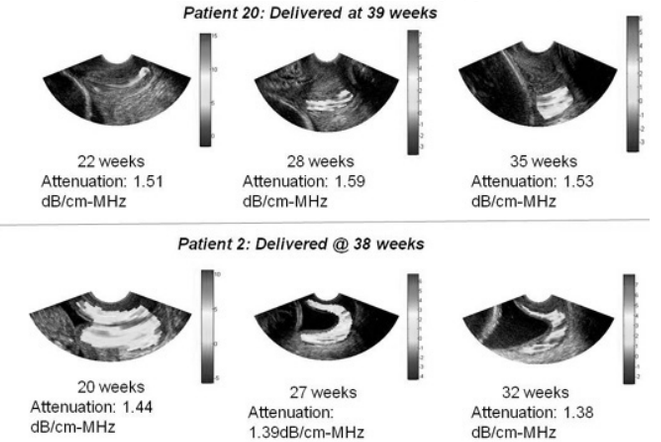
Ultrasonic Attenuation of the Human Cervix during Pregnancy. Barbara L McFarlin,[1] Viksit Kumar,[2] Andrew Nguyen,[2] Timothy A Bigelow.[2] *[1]Women Children and Family Health Science, University of Illinois at Chicago, Chicago, IL, USA; [2]Electrical and Computer Engineering, Iowa State University, Ames, IA, USA.*
Objectives: Attenuation quantifies anatomic microstructure and has been found to be related to cervix tissue stiffness, water content and collagen concentration. We sought to determine whether estimates of ultrasonic attenuation were related to cervical change in pregnancy.
Methods: 20 African American (AA) women consented to three serial transvaginal scans of the cervix using a 5- to 9-MHz transducer during pregnancy to estimate cervical length and attenuation. Ultrasound radio frequency data (RF) with corresponding beam-formed images were obtained with a Z.one (Zonare) ultrasound system. Attenuation was estimated by comparing the echoes from the cervices to those from a tissue-mimicking phantom. RF data were analyzed offline with Matlab.
Results: Of the 20 AA women, 17 delivered at term, 2 terminated due to anomalies, 1 delivered at 34 weeks. Attenuation was correlated with gestational age and weeks to delivery (p<0001); but not cervical length (p=.57). Cervical length was correlated with gestational age and weeks to delivery (p<.01). Of the women who delivered at term, the mean attenuation at 21 weeks was 1.57dB/cm-MHz; at 28 weeks was 1.36 dB/cm-MHz; and at 34 weeks was 1.17 dB/cm-MHz (p =.06). A woman who delivered at 34 weeks gestation, had an initial cervical length of 3.3 cm and attenuation of 0.68 dB/cm-MHz on the first scan at 23 weeks. One woman had marked cervical shortening and funneling but maintained a high attenuation value and delivered at 38 weeks (fig 1, patient 2). For the individual woman delivering at term, attenuation values maintained a constant value between the first and third scans (fig 1, patient 20).



Conclusions: These results suggest that ultrasonic attenuation is an objective noninvasive method to indicate cervix tissue property changes associated with cervical remodeling. Further research will be needed to determine whether ultrasonic attenuation can be a predictor of women destined to deliver at term or preterm. (Support: UIC CCTS NCRR UL1RR029879 and NIH R21HD06279)

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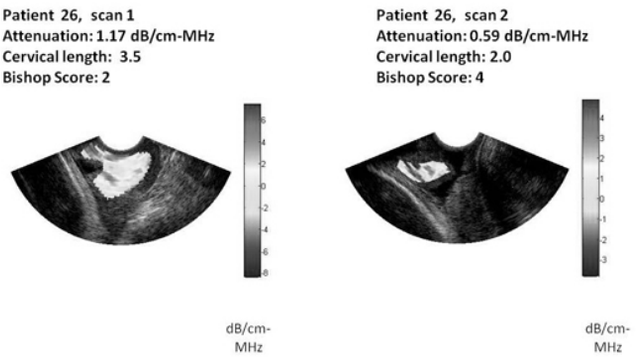
Ultrasonic Attenuation Detects Cervical Remodeling in Women Admitted for Induction of Labor. Barbara L McFarlin,[1] Jennifer Balash,[2] Viksit Kumar,[3] Andrew Nguyen,[3] Timothy A Bigelow,[3] Xavier Pombar,[2] Jacques S Abramowicz.[2] *[1]Women Children and Family Health Science, University of Illinois at Chicago, Chicago, IL, USA; [2]Obstetrics and Gynecology, Rush University, Chicago, IL, USA; [3]Engineering, Iowa State University, Ames, IA, USA.*
Objective: Pharmacological cervical ripening agents such as prostaglandins can accelerate the process of cervical ripening in a short amount of time. In our previous animal studies, ultrasonic attenuation was related to cervical remodeling (stiffness, collagen disorganization and water concentration) and preterm birth. The objective of this study was to determine whether ultrasound attenuation estimates can detect differences between pre- and post-medically induced cervical ripening in human pregnancy.
Methods: 20 women admitted for labor induction consented to a transvaginal scan of the cervix for attenuation estimation and cervical length with a 5- to 9-MHz transducer before cervical ripening and 12 hours later. Ultrasound radio frequency data (RF) with corresponding B-mode images were obtained with a Z.one (Zonare) ultrasound system. Attenuation was estimated by comparing the echoes from the cervices to those from a tissue-mimicking phantom. RF data were analyzed offline with Matlab.
Results: Cervical attenuation was correlated with hours to delivery (p=.03), but not Bishop score (p=.33) or cervical length (p=.44) or GA (p=.17).

Cervical characteristics

	PRE CERVICAL RIPENING	12 hr POST	p=
Attenuation (dB/cm-Mhz)	1.30	0.78	.0001
Cervix length (cm)	3.4	2.1	.0001
Bishop Score	3.3	6.6	.0001

Figure 1 displays a processed attenuation scan of the cervix of a women pre-cervical ripening and 12 hours later.



Conclusions: Attenuation is an objective measure of cervix tissue microstructural changes. Currently there is no method to objectively estimate cervical tissue property changes noninvasively. By realizing this ability, scientific interventions could be developed to modify term and preterm cervical ripening. Significant technological advances in computing and instrumentation have made it possible to improve ultrasound detection of cervical ripening. (Supported by NIH 1R21HD062790)

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Fetal Membrane Biomarker Network Induced by Intraamniotic Pathogens: Diversity and Mechanisms. Ramkumar Menon,[1] Geeta Bhat,[1] George Saade,[1] Stephen J Fortunato,[2] Morgan R Peltier.[3] *[1]Ob & Gyn, The University of Texas Medical Branch at Galveston; [2]The Perinatal Research Center, Centennial Women's Hospital; [3]Clinical Obstetrics, Gynecology and Reproductive Medicine, Winthrop University Hospital.*

OBJECTIVE: Fetal immune responses to bacteria associated with intra-amniotic infection (IAI) vary between pathogens. To better understand the downstream pathophysiologic pathways that ultimately result in preterm birth (PTB) and their influence by amniotic fluid (AF), we studied dysregulated biomarkers from normal term fetal membranes exposed to IAI pathogens using Ingenuity Pathway Analysis (IPA).

METHODS: Fetal membranes from women (n = 11) at term, not in labor undergoing Cesarean sections were placed in an organ explant culture system. Membranes were stimulated with *Ureaplasma urealyticum (UU)*, *Ureaplasma parvum (UP)*, *Mycoplasma hominis (MH)*, *E. coli (EC)*, Group B *Streptococci* (GBS), *Polyporhans gingivalis (PG), or Gardnerella vaginalis (GV)* in the presence or absence of 50 % (v/v) autologous AF. Data for biomarkers (IL1β, IL2, IL6, IL8, IL10 and TNFα) that were dysregulated between stimulated and unstimulated tissues were analyzed using IPA. Network interactions and pathway specificity identified by IPA were evaluated by Fisher's exact test.

RESULTS: Fetal membrane response to EC, PG and GBS was profoundly affected by AF (P<0.0001). In the absence of AF, lipid metabolism (EC), antigen presentation (PG) and chemotaxis (GBS) were the predominant pathways. Membranes stimulated with AF pathways were inflammation (EC), chemotaxis (PG) and antigen presentation (GBS), respectively. AF had no effect on molecular interactions of cultures stimulated by GV and genital mycoplasmas whose underlying pathways were defined by markers of altered lipid metabolism and balanced inflammatory responses, respectively (P=0.0001).

CONCLUSION: IPA analysis suggested that differences in the fetal membrane cytokine response to various pathogens documented different networks that define underlying IAI pathophysiology. Inflammation is the underlying mechanisms with most pathogens but the network of inflammatory biomarkers and the type of immune response produced by each pathogen is different suggesting that PTB pathways are not generalizable and interventions should be tailored and directed towards specific pathways. AF, a source of antimicrobial factors, influences fetal immune response to bacteria. Autologus AF should be a part of *in vitro* experimental setups.

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Bivalent Epigenetic Modifications Regulate PTGS2 Expression during Gestation in the Amnion. Carolyn M Mitchell,[1,3] Sze Chai,[1,3] Gemma Madsen,[1,3] Jonathan J Hirst,[1,4] Tamas Zakar.[1,2,3] *[1]Mothers and Babies Research Centre, University of Newcastle, NSW, Australia; [2]Obstetrics and Gynaecology, John Hunter Hospital, New Lambton Heights, NSW, Australia; [3]School of Medicine and Public Health, University of Newcastle, NSW, Australia; [4]School of Biomedical Science, University of Newcastle, NSW, Australia.*

Increasing PTGS2 expression occurs in the amnion with advancing gestation; however the mechanisms regulating this increase are unknown. "Bivalent" genes contain active and repressive histone modifications which can influence gene expression. We believe that bivalent epigenetic histone modifications repress PTGS2 expression in the amnion early in pregnancy. At term repressive modifications are removed and permissive modifications increase leading to gene activation and labor. We determined the pattern of active and repressive histone modifications at the PTGS2 gene in early and late pregnancy.

Amnion was collected in early pregnancy (16-18 weeks) and at term after elective caesarean section or following spontaneous labor. Chromatin immunoprecipitation (ChIP) was performed with antibodies to the activating epigenetic modifications histone H3 tri-methyl K4 (H3K4me3), acetylated histones-3 (acH3) and -4 (acH4), histone H3 tri-methyl K36 (H3K36me3) and the repressive histone marks histone H3 tri-methyl K9 (H3K9me3) and H3 tri-methyl K27 (H3K27me3). Analysis of the immunoprecipitated DNA was by quantitative real time PCR using 12 primer pairs spanning the PTGS2 gene. The H3K4me3 distribution peaked in the promoter region near the transcriptional start site. H3K4me3 levels were low early in gestation and increased significantly at term ($p < 0.005$). H3K27me3 spanned the promoter and the transcribed region of the gene with levels decreasing around the TATA site. H3K27me3 levels were high early in gestation and decreased at term. The ratio of H3K4me3 to H3K27me3 increased significantly with advancing gestation ($p<0.001$). Both acetylated histones were low early in gestation with acH3 rising significantly at term before labor ($p<0.041$) and acH4 rising after labor ($p<0.002$). H3K9me3 and H3K36me3 were present on the PTGS2 gene but did not change with advancing gestation.

Thus, the PTGS2 gene is bivalently marked with both H3K27me3 and H3K4me3 histone modifications. The changes with advancing gestation are consistent with a switch from a repressive to a permissive chromatin structure suggesting that the PTGS2 gene is activated by epigenetic mechanisms at term.

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Fetal Fibronectin Extra Domain-A (EDA): Pathogenesis of Preterm Labor and Preterm Rupture of Membranes. Haruta Mogami, Annavarapu H Kishore, Haolin Shi, Patrick W Keller, R Ann Word. *OB & GYN, UT Southwestern Medical Center, Dallas, TX.*

Objectives

Fibronectin (Fn) is a multidomain protein. These domains are involved in the assembly of Fn into a multimeric fibrillar matrix. Fetal Fn (fFn) is a unique Fn with an alternatively spliced exon encoding extra domain-A (EDA). Previously, we found that fFn activated MMPs and COX-2 in mesenchymal cells of the amnion suggesting that fFn is not only a marker of preterm labor but also plays a functional role in the pathogenesis of preterm labor. In this study, we investigated the functional roles of EDA of fFn in matrix remodeling and prostaglandin (PG) synthesis of amnion.

Results

Treatment of human amnion mesenchymal cells with fFn (80nM,24h) resulted in increases in *MMP1* (18-fold,P<0.01), *MMP9* (4-fold,P<0.01), and *COX2* (8-fold,P<0.01) mRNA. Interestingly, purified plasma Fn (pFn,80nM,24hr) did not affect expression of these genes. The proportion of EDA-containing Fn mRNA was significantly increased in amnion (38±3%) relative to deciduas (13±4%,P<0.01), and increased further with TNF-α treatment (10ng/ml,24h) to 57±4%. Immunoblot analysis with an antibody specific for EDA demonstrated strong immunoreactivity with fFn which was absent in pFn. Treatment of mesenchymal cells with recombinant EDA (100nM,24hr) resulted in increases in *MMP1* (280-fold,P<0.01), *MMP9* (20-fold,P<0.01), and *COX2* (4-fold,P<0.01) mRNA as well as PGE_2 (from 21 to 606 pg/mg protein,P<0.01), whereas recombinant III_{11} (ctl Fn domain,100nM,24h) had no effect. Although unaffected by β1 integrin antibodies, Toll-like receptor-4 (TLR-4) neutralizing antibody inhibited fFn- and EDA-induced increases in *MMP1* and *COX2* mRNA significantly (67 and 87%, respectively). The pathophysiologic significance of free fFn was confirmed in vivo by injection of fFn or control at the maternal-fetal interface in pregnant mice on gestation d17. Whereas control-injected mice delivered at term, 6 of 9 fFn-injected mice delivered prematurely (23±2h,P<0.02,χ^2).

Conclusion

EDA was identified as a pathological functional domain of fFn, increasing MMPs and PGE_2 in amnion via activation of TLR-4 in mesenchymal cells. The release of EDA-fFn from its matrix environment results in increased collagenase activity thereby leading to rupture of membranes. Furthermore, EDA-induced PG synthesis may facilitate cervical ripening, myometrial contractions. Neutralization of fFn-EDA or antagonism of TLR-4 in amnion may have therapeutic potential for preterm labor and PROM.

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Direct Permeability Measurements of Pregnant and Nonpregnant Human Cervical Tissue. Michael Fernandez,[1] Joy Vink,[2] Ronald Wapner,[2] Jan Kitajewski,[2] Claire Reeves,[2] Kristin Myers.[1] *[1]Engineering, Columbia University, New York, NY, USA; [2]Columbia University Medical Center, New York, NY, USA.*

Objective: Studies on the mechanical properties of human cervical tissue are limited. Permeability is a *material* property that characterizes the ability of fluid to flow through a porous structure. For collagenous tissues, a high permeability is associated with disorganized collagen morphology and a compliant material. Our aim is to develop a reliable method to directly measure the permeability of cervical tissue and to report this value for the first time for pregnant (PG) and non-pregnant (NP) specimens.

Methods: Disk-shaped stroma specimens (diameter = 8mm, height = 1-2mm) were taken post hysterectomy. 3 specimens from 2 PG and 8 specimens from 2 NP patients were collected. Specimens were thawed and equilibrated in 0.15 M NaCl, and permeability was measured using a custom setup (Fig.A). Specimens were subjected to a fluid pressure gradient parallel to the inner canal, and the fluid flow rate through the tissue was measured. Permeability was calculated using Darcy's law. PG and NP specimens were also subjected to confined compression stress-relaxation tests to 5, 10, and 15% strain using previously published methods.

Results: Averaged permeability for PG (n=3) and NP (n=8) cervical tissue was 0.73±0.44E-12 and 0.033±0.02E-12 $m^4/(N \cdot s)$, respectively. Values for each patient (Fig.B) were consistent in magnitude between specimens, verifying the repeatability of our mechanical testing method. Preliminary compression tests showed that the higher PG permeability was associated with a more compliant material (Fig.C).

Conclusion: We report the first direct permeability measurements of PG and NP human cervical tissue. PG tissue permeability was an order of magnitude larger than NP. These results correlate with our previously published findings that PG tissue has a more dispersed collagen structure resulting in greater compliance. Low patient trial numbers prevented the statistical comparison of PG and NP specimens. Additional samples are being evaluated to test the hypothesis that permeability increases during pregnancy.

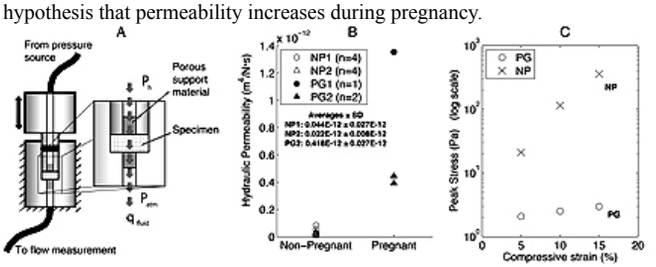

Figure A) Schematic of permeability test showing flow path and specimen location. **B)** Scatter plot of cervical tissue permeability for non-pregnant (NP1 & NP2) and pregnant (PG1 & PG2) patients. Considering the full scale of readings, there is significantly low variation between specimens from the same cervix. **C)** Typical confined compression peak stress values for pregnant (PG) and non-pregnant (NP) specimens under a 5, 10 and 15% strain ramp-hold loading regimen. The NP response dominates the graph, indicating lower permeability and therefore higher resistance to mechanical deformation.

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Anthrax Toxin Receptor 2 Knock-Out and Wild-Type Mouse Cervix Exhibit Time-Dependent Mechanical Properties. Kyoko Yoshida,[1] Claire Reeves,[2] Jan Kitajewski,[2] Ronald Wapner,[2] Joy Vink,[2] Kristin Myers.[1] *[1]Mechanical Engineering, Columbia University, USA; [2]Obstetrics and Gynecology, Columbia University Medical Center, USA.*

Objective: Anthrax Toxin Receptor 2 (*Antxr2*) knock-out (KO) mice exhibit an abnormality in the extracellular matrix (ECM) maintenance of the cervix, resulting in an over-accumulation of collagen and the failure of the cervix to ripen for delivery. Therefore, we hypothesize that cervical tissue from KO animals are less compliant than the wild-type (WT). Our objective is to quantify and compare the time-dependent mechanical properties of KO and WT cervices.

Methods: 2-3 month old nulliparous nonpregnant KO (n=4) and WT (n=4) cervices were isolated, attached to tensile grips of a material tester, and kept hydrated in a saline bath (Fig.1A,B). After swelling equilibration, a constant tensile force was applied to the tissue while creep deformation was continuously recorded with a non-contacting video extensometer and the material tester. The stress-strain relationship was calculated based on specimen geometry, force, deformation, and boundary conditions. A two-time constant Kelvin-Voigt viscoelastic material model was fit to the experimental data, and the long-term creep compliance *D* was compared between the KO and WT specimens.

Results: Both nonpregnant KO and WT cervical tissue had a time-dependent mechanical response to a hold in tensile stress, evident by the continuous creep deformation over time (Fig.1C). On average, the KO nonpregnant samples were less compliant compared to age-matched WT specimens. However, there was no statistical difference between the two groups (Student's t-test, $p>0.05$), with the KO samples having a significant variation in *D*.

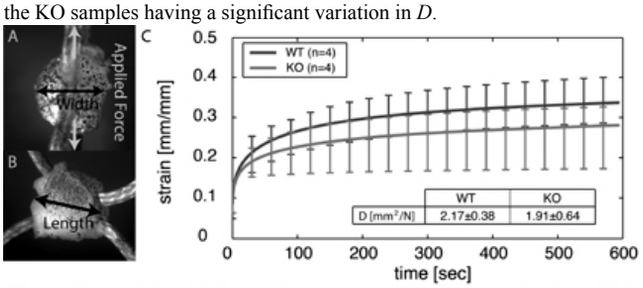


Figure 1: (A) Internal Os and (B) External Os of a KO mouse after swelling equilibration and before applying tensile foce. (C) averaged creep response for both *Antrx2+/+* and *Antrx2 -/-* cervix. Error bars indicate range of responses. The table shows the mean ± standard deviation of the long term creep compliance for WT and KO mice

Conclusion: The large spread in the KO creep response suggests that there may be inconsistencies in the ECM of the 2-3 month old KO mice from sample to sample, possibly due to the young age and limited estrus cycles. Additional mechanical studies are being conducted on older nonpregnant animals as well as gestation-timed pregnant KO and WT specimens to determine the role of *Antxr2* in ECM remodeling and cervical ripening.

F-245

Risk of Preterm Delivery after Treatment for Cervical Intraepithelial Neoplasia. Donald Peebles,[2] Alejandra Castanon,[1] Peter Brocklehurst,[2] Heather Evans,[3] Naveena Singh,[4] Patrick Walker,[3] Julietta Patnick,[5] Peter D Sasieni.[1] *[1]Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, QMUL, London, United Kingdom; [2]Institute for Women's Health, University College London, London, United Kingdom; [3]Department of Gynaecology, Royal Free Hampstead NHS Trust, London, United Kingdom; [4]Division of Cellular Pathology, Barts and the London NHS Trust, London, United Kingdom; [5]Director's Office, NHS Cancer Screening Programmes, Sheffield, United Kingdom.*

Background: There is a growing body of evidence linking treatment for cervical disease with an increased risk of a preterm delivery. We conducted a record linkage study in England to estimate the association between preterm births and treatment for cervical intraepithelial neoplasia.

Methods: Women were identified from clinical records in 12 English hospitals as having had cervical histology between 1987 and 2009. These women were linked by HES (Hospital Episode Statistics) to hospital obstetric records between 1998 and 2009 for the whole of England to identify live births whether prior to or subsequent to the histological sample. NHS maternity statistics (published by HES) from 2000/01 to 2009/10 were pooled to obtain an average population preterm delivery rate for the period.

Results: We identified 18527 births (in 13,218 women) with known gestational age of which 1723 (9.3%) were preterm (20-36 weeks). After excluding multiple births (twin and triplets) and births in women with a prior preterm delivery, there were 17980 births of which 1438 (7.99%) were preterm. The preterm delivery rate in England was 6.7%. The preterm risk ratio comparing births after histology (n=13931) to those prior to histology (n=4049) was 1.07 (95% CI 0.94-1.21). Among 11802 births after histology with known sample type (biopsy or cone/loop excision), the preterm risk was slightly higher in those with a cone/loop: risk ratio 1.10 (95% CI 0.97-1.25). The risk ratio among those who subsequently had a cone/loop compared to those who subsequently had a biopsy was 1.24 (0.94-1.63).

Conclusions: This study suggests that the risk associated with treatment of CIN in many studies does not apply to the treatment as carried out by the NHS Cervical Screening Programme in England. A future study will obtain treatment details in a nested case-control study.

F-246

Cervical-Vaginal Fluid Measurements of Potential Biomarkers: Sampling Variation with Blind Vaginal Swab Compared to Sterile Speculum Examination. Jon Larrabee,[1] Amanda Kinhnarath,[1] Janice Snyder,[1] Monica Rincon,[1] Rene Riano,[2] Leonardo Pereira.[1] *[1]OBGYN, Oregon Health & Science University, Portland, OR, USA; [2]OBGYN, Flushing Medical Center, Flushing, NY, USA.*

Objective: Proteomic analysis of amniotic and cervical-vaginal fluid (CVF) during intra-amniotic infection and threatened preterm birth (PTB) has identified Calgranulin B (Cal B), Matrix Metalloproteinase 9 (MMP-9) and L-Plastin as proteins of significance. These proteins are highly abundant in CVF, suggesting a possible role for them as diagnostic biomarkers. Our objective was to determine the variation in Cal B, MMP-9 and L-Plastin concentrations in CVF when sampled by "blind" swabbing of the posterior vagina compared to collection during sterile speculum exam (SSE).

Study Design: A prospective cohort of patients with singletons, threatened PTB, and intact membranes underwent CVF sampling by trained providers using standard technique: blind swab was collected by attempted placement into the posterior vaginal fornix and rotation for 20 seconds followed by SSE and swabbing the posterior vaginal fornix for 20 seconds under direct visualization. All subjects had not had intercourse, digital vaginal exam, or transvaginal sonogram in the prior 24 hours. Western immunoblotting was used to compare Cal B, MMP-9 and L-Plastin levels between blind and SSE samples.

Results: Twenty women were recruited at a mean of 27.2 weeks gestation. MMP-9 was present in only 3/20 subjects, however the levels appeared to be consistent between the 2 sampling methods (Fig 1). Cal B was present in all patients, but levels were inconsistent between methods in 6/20 subjects. L-Plastin was detected in 14/20 subjects, but demonstrated the greatest variability by sample collection method.

Conclusion: Blind sampling offers an easier, less invasive method for collecting CVF. While some biomarkers, such as MMP-9, can potentially be collected by blind vaginal swab, the reproducibility of each potential biomarker needs to be prospectively validated since several candidate proteins, such as Cal B and L-Plastin, demonstrate great variation in levels by sample collection method utilized.

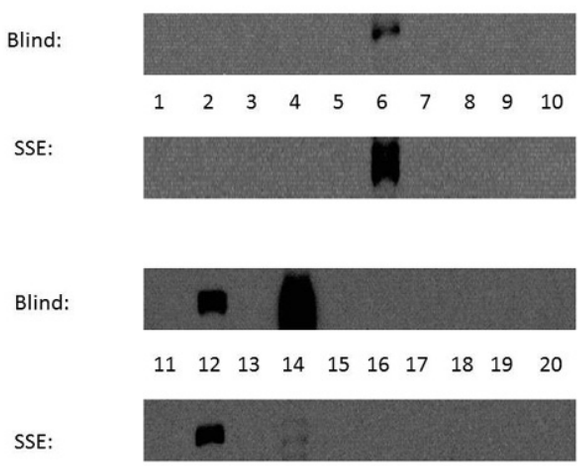


Figure 1: MMP-9 Western Blot, Blind vs SSE CVF samples

F-247

Thrombin Weakens the Amnion Directly Rather Than through Its Receptors. M Puthiyachirakkal,[1] D Kumar,[1] RM Moore,[1] E Philipson,[2] BM Mercer,[3] JM Mansour,[4] JJ Moore.[1,3] *[1]Pediatrics, MetroHealth Medical Center, CWRU; [2]Women's Institute, Cleveland Clinic; [3]Reproductive Biology, CWRU; [4]Mechanical and Aerospace Engineering, CWRU.*

Although preterm premature rupture of fetal membranes (pPROM) is a major cause of preterm births, the mechanisms mediating the fetal membrane (FM) weakening and eventual pPROM are poorly understood. Abruption-associated increased thrombin production and infection-inflammation are postulated to play major roles in pPROM. We have previously demonstrated, utilizing our *in vitro* model system, that both thrombin and inflammatory cytokines (TNFα and IL-1β) remodel and weaken amnion. While further exploring the mechanisms of FM weakening we have shown that thrombin can weaken isolated amnion but cytokines weaken amnion indirectly by initially interacting with choriodecidua and releasing an, as yet, unidentified soluble activator(s).

The purpose of this study was to determine whether thrombin weakens isolated amnion by direct action on the extracellular matrix and/or through protease activated receptors (PARs 1,2,3,4).

PARs expression in amnion and primary amnion cells was determined by immunohistochemistry and Western Blot. Isolated amnion from FM obtained following unlabored repeat cesarean section were devitalized by exposure to UV radiation (254nm) for one hour and three subsequent freeze-thaw cycles. Absence of viable cells in the amnion was confirmed by LDH assay. Devitalized amnion explants were incubated with thrombin, with or without its specific inhibitor hirudin. Subsequent rupture strength (breaking force) was determined using our published methodology. MMP 2 was determined by zymography and western blot.

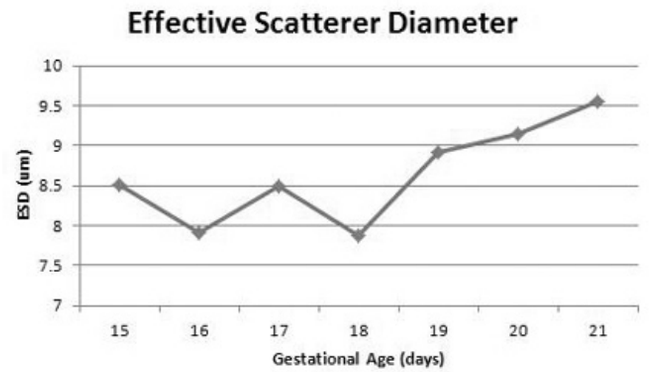
Primary amnion cells, isolated amnion, or amnion after oxidative stress did not express PAR1, 2, 3 or 4 by immunohistochemistry or Western Blot. Thrombin (T, 0-100U/ml) induced dose-dependent weakening [control (7.64±2.58N); T10 (5.06±2.72 N, p 0.003); T50 (2.30±0.88 N, $p<0.001$);T100 (3.36±1.74, $p<0.001$)] of devitalized amnion pieces. Thrombin converted pro-MMP2 to active MMP2 at all doses. Preincubation with hirudin (H) prevented the thrombin-induced weakening of devitalized amnion at the lowest (T10) tested thrombin dose [control (7.64±2.58N); T10 (5.06±2.72 N, $p=0.011$); H+T10 (8.95±1.14 N, $p=0.35$)].

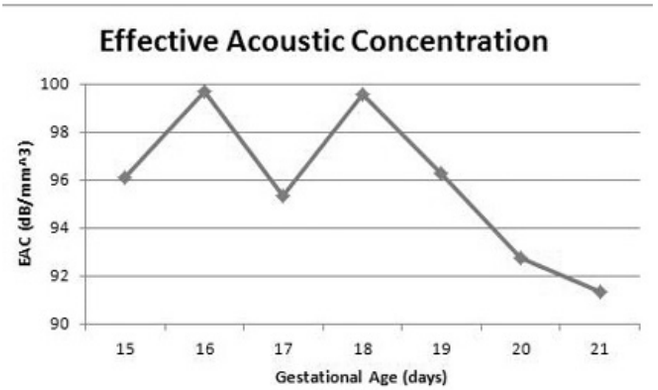
In summary, rather than via PAR receptor activation, thrombin appears to weaken the amnion by direct action on the extracellular matrix converting stored MMPs, including MMP2 to their active form.

F-248

Quantitative Ultrasound Parameter Estimation of the Rat Cervix as a Function of Gestational Age. Ellora Sen-Gupta,[1] Barbara L McFarlin,[2] Timothy A Bigelow,[3] Edward K Chien,[4] William D O'Brien, Jr.[1] *[1]Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, USA; [2]Department of Women, Children, and Family Health Science, University of Illinois at Chicago, Chicago, IL, USA; [3]Department of Electrical and Computer Engineering, Iowa State University, Ames, IA, USA; [4]Alpert Medical School of Brown University, Women & Infants Hospital of Rhode Island, Providence, RI, USA.*

Preterm birth is associated with early ripening of the cervix. Quantitative ultrasound (QUS) can be used as a safe and noninvasive method to monitor microstructural tissue changes in the cervix tissue associated with cervical remodeling throughout pregnancy. The goal of this research is to assess rat cervical remodeling using two QUS parameters: the effective scatterer diameter (ESD) and the effective acoustic concentration (EAC), the latter being sensitive to both the strength of the scattering source and number of scattering sources in the tissue. A sample size of 150 Sprague-Dawley timed-pregnant rats were scanned between two to five times throughout the pregnancy with a 40-MHz endovaginal transducer while under isoflurane inhalation anesthesia. Scans of the cervix were taken with the focus of the transducer centered within the cervix tissue. The radio frequency data from the scans was acquired, processed in Matlab, and modeled using the fluid-filled sphere form factor. Preliminary analysis of 40% of the sample set shows that as a function of gestational age from 15 to 21 days (rats typically deliver on day 21), the ESD generally increased from 8.5 to 9.6 microns and the EAC generally decreased from 96.1 to 91.3 dB/mm^3.





The ESD's increase was minimal suggesting that the scatterer remained mostly unchanged. However, the EAC's decrease suggests that the "concentration" of scatterers decreased, consistent with an increase in fluids as the cervix remodels. QUS parameters demonstrate the ability to detect microstructural changes in the rat cervix during pregnancy, and may lead to more accurate predictions of birth. [Support: R21HD058705]

F-249

Electrical Stimulation (ES) of the Cervix during Pregnancy Produces Early Cervical Ripening. Shao-Qing Shi, Leili Shi, Robert E Garfield. *Ob/Gyn, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA.*

Cervical ripening is required for successful delivery and involves assorted pathways that are associated with inflammation. Various treatments are used clinically to ripen the cervix when appropriate to induce delivery. **Objective:** To determine if ES of the cervix will produce ripening during pregnancy. **Study Design:** Timed-pregnant Sprague-Dawley rats (n = 34) were used. On day 15 of gestation an electrode probe (Grass Instruments, West Warwick, RI, model F-BSE1) was placed on surface of the cervix. In some animals the probe was placed in the animals but no current was applied (controls). In other animals current pulses (100 microamps, 10 pulses/sec, and 20 msec/pulse) were applied (Grass stimulator, model S88) through electrodes for 2 hours. Daily measurements of cervical light-induced florescence (LIF, photon counts of collagen x-bridge fluorescence) were made on days 16 of gestation and daily until spontaneous delivery (day 22) to estimate changes in cervical ripening. After ES the cervix of all animals was examined for damage with a small endoscopic camera. Delivery times, fetal weights, and viability were made following delivery of both control and ES-treated animals. Statistical differences were assessed by one-way ANOVA and the Student's t-test. **Results:** In control animals the cervical LIF values (mean photon counts ± SD) slowly and significantly ($P<0.05$) decline from day 15 of gestation (2582 ± 88) and reach the lowest levels during delivery (732 ± 140). LIF values are significantly lower in ES animals on days 16 and 17 of gestation as compared to controls (day 16: 663 ± 87 vs. controls, 1968 ± 145, $P<0.001$; day 17: 833 ± 24 vs. controls, 1677 ± 140, $P<0.001$) and remain low until delivery. Control and ES animals deliver normally on day 22 of gestation and all fetuses are normal and healthy. Fetal weights (g ± SD) are not significantly ($P>0.05$) different between control animals (6.28 ± 0.06) compared to ES animals (6.12 ± 0.58). **Conclusions:** 1) ES of the cervix, well below perception levels, offers a unique method to ripen the cervix without pain or damage; 2) ES does not produce early birth; 3) Optimal ES parameters remain to be defined; 4) ES may be useful for treatment of pregnant patients in combination with uterotonic agents; 5) The mechanism for ES ripening remains unknown but may include activation of neural or inflammatory pathways.

F-250

Distribution and Up-Regulation of H6PD Expression by Glucocorticoids in Human Fetal Membranes. Weihua Wang,[1] Wangshen Wang,[1] Jianneg Li,[1] Chunming Guo,[2] Leslie Myatt,[2] Kang Sun.[1,2] *[1]School of Life Sciences, Fudan University, Shanghai, China; [2]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Glucocorticoids play important roles in fetal development and parturition. Toward the end of gestation, human fetal membranes express abundant 11β--hydroxysteroid dehydrogenase type 1 (11β-HSD1) which regenerates biologically active cortisol from inert cortisone and provides an extra-adrenal source of cortisol. However, 11β--HSD1 is a bi-directional oxo-reductase in vivo and its reductase activity that regenerates cortisol from cortisone depends on the availability of the co-factor NADPH which derives from the enzymatic activity of hexose-6-phospho-dehydrogenase (H6PD). Knock-out of the H6PD gene reverses the enzymatic activity of 11β--HSD1 to oxidase, suggesting the crucial role of H6PD in determining the catalytic direction of 11β--HSD1. We have demonstrated previously that glucocorticoids positively feedback on 11β--HSD1 expression in human fetal membranes, which may play a crucial role in the feed-forward production of cortisol and prostaglandins in the fetal membranes at the end of pregnancy. Considering the role of H6PD in 11β--HSD1 catalytic activity, we hypothesize that there is concurrent induction of H6PD and 11β--HSD1 by glucocorticoids in the fetal membranes. In this study we compared the distribution of H6PD and 11β--HSD1 in human fetal membranes obtained from pregnant women at c section at term and investigated the effect of cortisol on the expression of H6PD and 11β--HSD1 in cultured primary amnion fibroblasts. Immunohistochemical staining revealed that the distribution of H6PD and 11β--HSD1 in the fetal membranes was strikingly similar. Immunostaining for both H6PD and 11b-HSD1 was found in the amnion epithelial cells and fibroblasts, chorionic trophoblasts as well as decidual cells. Cortisol (0.01-1μM) treatment of the amnion fibroblasts significantly induced both H6PD and 11β--HSD1 mRNA and protein expression in a concentration-dependent manner, which could be blocked by glucocorticoid receptor antagonist RU486. In conclusion, the parallel distribution of H6PD and 11β--HSD1 and concurrent induction of H6PD and 11β--HSD1 by glucocorticoids may be prerequisite for the increased reductase activity of 11β--HSD1 in the fetal membranes.

F-251

Differential Role of Prostaglandins in Term Versus Infection or Progesterone Withdrawal-Induced Preterm Cervical Ripening. Brenda C Timmons,[1] Jeff Reese,[2] Noah Ehinger,[2] BC Paria,[2] Mala Mahendroo.[1] *[1]OBGYN, UT Southwestern Medical Center, Dallas, TX, USA; [2]Pediatrics, Vanderbilt University, Nashville, TN, USA.*

Objective: While exogenous administration of prostaglandin (PGs) to women promotes cervical remodeling, it's unclear if endogenous PGs are necessary for term cervical ripening. In this study, we used mouse models to evaluate the role of cervical PGs during term and preterm ripening.

Methods: Two preterm birth models were evaluated along with sham controls. Infection-induced premature ripening was evaluated 1-6h post intrauterine LPS while progesterone withdrawal-induced preterm ripening was evaluated 3-12h after RU486. Cervical expression of Cox1/2, PG receptors, PGE synthase (PGES), and PG dehydrogenase (PGDH) were evaluated by QPCR. COX1/2 localization was examined by IHC and in situ hybridization. Cervix PG levels were measured by gas chromatography/mass spectrometry.

Results: Throughout pregnancy, transcripts encoding Cox1/2 were low with a modest but significant increase postpartum (PP). Additionally, there was no increase in PGE2, PGF2a, PGD2, 6KPGF1a and TXB2 in the term or PP cervix. In contrast, Cox 1 expression increased 2-3 fold by 12h post RU486 while Cox 2 increased 2h after LPS treatment and was induced 60 fold by 6h. PGE2, PGF2a, PGD2, and 6KPGF1a levels were induced in the LPS-treated cervix compared to gestation d15, d18, PP and RU486-treated animals. The rise in local PG synthesis was further supported by an upregulation of PGES and a decline in PGDH in the LPS model. PG receptors (EP1-4) were upregulated PP but not in the preterm models with the exception of EP1 with RU486 treatment.

Conclusion: While PG application is routinely used to induce cervical remodeling in women, studies analyzing cervical mucus samples report no increase in PG concentrations during ripening . Studies to determine the

necessity of PGs in term and preterm ripening were carried out in mice as the process of remodeling is similar to human. Gene expression and PG levels suggest that neither local PG synthesis nor PGs derived from other tissues are required for term ripening. The PP increase in PG receptors suggest increased PG function during PP repair. Mechanisms of term and preterm ripening differ. Increased local PG synthesis and reduced metabolism are unique to infection-mediated preterm ripening. Thus, PGs have a role in infection-induced cervical ripening but may not be necessary for the remodeling process at term.

F-252

A Progesterone Receptor-Mediated Mechanism Delays Preterm Remodeling of the Cervix and Premature Birth in Mice. Steven M Yellon, Kathleen N Stutz, Jaclyn Cooperrider, Michael A Kirby. *Physiology, Human Anatomy&Pathology, Pediatrics, and the Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Background Withdrawal of progesterone (P4) promotes premature birth and inflammatory processes associated with remodeling of the cervix. Inflammation-induced preterm remodeling of the cervix and premature birth is blocked by medroxyprogesterone acetate (MPA), a receptor agonist to P4 (PR) and glucocorticoids (GR) (*Reprod Sci 16:257,2009*). This study tested the hypothesis that a PR-dependent mechanism mediates the effects of progestational agents to block preterm cervical remodeling induced by loss of systemic P4. **Methods** Pregnant CD-1 mice were Sham-operated or ovariectomized (Ovx) on day 16 postbreeding. Ovx mice received vehicle (V), GR agonist (dexamethasone, Dex), or progestational treatments, i.e., gonadal steroids (E2+P4 silastic capsules, P4 for 2 days), R5020 (pure PR agonist), or MPA. Mice were killed before or after birth and the cervix analyzed for cell density, collagen, and resident immune cells as before (*Biol Reprod 85:498,2011*). **Results** Sham mice gave birth by day 19 postbreeding while Ovx mice given V or Dex delivered preterm (<24h, day 17). By contrast, pups were born at normal term or later in Ovx mice given progestational agents. By the day before birth, the cervix of Sham controls had fewer cell nuclei/area and less collagen. Cervical hypertrophy and collagen degradation was not advanced with preterm delivery in Ovx (V or Dex groups) and not promoted by P4, R5020, or MPA. More macrophages were in the cervix by the day before birth in Sham, Ovx+V, and Ovx+Dex groups (vs Sham D15). Only the PR agonist R5020 blocked M0 recruitment. Neutrophils (Neu) were increased in the cervix of Sham mice by the day before birth, but numbers remained low in all Ovx groups regardless of treatment. **Conclusions** These findings indicate that increases in both macrophages and Neu are characteristics of cervical remodeling in Shams before term. By contrast, systemic P4 withdrawal-induced preterm birth did not promote remodeling or Neu recruitment. Evidence that the pure PR agonist prevents both preterm macrophage recruitment and premature birth suggests that a PR-dependent mechanism may regulate similar remodeling processes at term. Thus, PR regulation of inflammatory processes may be useful to forestall preterm remodeling of the cervix and premature birth. Supported by NIH HD054931.

F-253

Using 3T Magnetic Resonance Imaging To Assess Fat Distribution and Ectopic Lipid Deposition in Morbidly Obese Pregnant Women in the Third Trimester. Sarah M Barr,[1] Carolyn Chiswick,[1] Annette Cooper,[2] Calum Gray,[2] Scott Semple,[2] Nicholas M Morton,[3] Brian R Walker,[3] Jane E Norman.[1] *[1]Tommy's Centre for Maternal and Fetal Health, MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Midlothian, United Kingdom; [2]Clinical Research Imaging Centre, University of Edinburgh, Edinburgh, Midlothian, United Kingdom; [3]Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, Midlothian, United Kingdom.*

Maternal obesity affects nearly 30% of pregnancies in the UK and is associated with increased maternal & fetal morbidity and mortality, eg. gestational diabetes and pre-eclampsia. In non-pregnant obese subjects, obesity is associated with intra-adipose insulin resistance, reduced capacity of adipose tissue for lipid storage, and ectopic lipid deposition in liver and skeletal muscle, contributing to insulin resistance at these sites. In pregnancy, a progressive insulin resistant state, we hypothesize that morbid obesity will be associated with enhanced ectopic lipid accumulation in the third trimester.

Healthy lean (BMI<25kg/m^2) and morbidly obese (BMI>40kg/m^2) women were imaged at approximately 36 weeks gestation in a 3T MRI scanner (Siemens) (n=10/group). ^{1}H-Magnetic Resonance Spectroscopy was carried out in the right quadriceps muscle using a voxel size of 2cm^3 and lipid concentration calculated from the water-suppressed acquisition using the spectroscopy analysis tool JMRUI. Intrahepatic lipid signal contribution was calculated through subtraction of in and out of phase images. Subcutaneous and intra-abdominal adipose tissue distribution was quantified using a semi-automated thresholding technique (SliceOMatic) on a 20x2mm-slice region of abdomen cranial to the left renal hilum.

MRI imaging at 3T was well tolerated by all women. At 36 weeks, hepatic lipid content was low and there was no significant difference between lean and obese pregnant women (1.6±0.35 vs 1.2±0.3%). Subcutaneous adipose tissue was the largest abdominal adipose tissue depot in lean and obese women. Obese women had significantly greater subcutaneous (2.3 vs 0.5kg, p<0.0001), intra-abdominal (0.5 vs 0.1kg, p<0.0001) and intra-muscular (57.1 vs 6.7g, p=0.005) adipose tissue depots compared with lean women.

We conclude that whilst obese women have significantly greater adipose tissue deposition than lean women, this is not associated with elevated hepatic lipid accumulation in the third trimester and points to a potential hepato-protective mechanism in pregnancy.

F-254

Anxiety and Depressed Mood in Pregnant Obese Women: A Prospective Controlled Cohort Study. Annick Bogaerts,[1] Roland Devlieger,[2] Erik Nuyts,[3] Ingrid Witters,[4] Wilfried Gyselaers,[5] Isabelle Guelinckx,[6] Bea Van den Bergh.[7] *[1]Healthcare Research, PHL-KHLim University College, Hasselt, Belgium; [2]Obstetrics & Gynaecology, University Hospitals of KULeuven, Leuven, Belgium; [3]Healthcare Research, PHL University College, Hasselt, Belgium; [4]Prenatal Diagnosis, East Limburg Hospital, Genk, Belgium; [5]Obstetrics, East Limburg Hospital, Genk, Belgium; [6]Public Health Nutrition, KULeuven, Leuven, Belgium; [7]Psychology, Tilburg University, Tilburg, Netherlands.*

Background: Psychological health in obese women throughout pregnancy has been poorly studied. The objective of this study is to compare levels of anxiety and depressed mood during pregnancy in obese versus normal-weight women.

Methods: Sixty-three pregnant obese women with a mean body mass index of 34.3(4.1) kg/m² and 156 normal weight controls with a mean body mass index of 22.0(1.4) kg/m² were included prospectively before 15 weeks of gestation. All women received standard care during pregnancy. Levels of state and trait anxiety (STAI) and depressed mood (EDS) were measured during the first, second and third trimester of pregnancy. A linear mixed-effect model with repeated measures was used to evaluate group differences, controlling for interference from socio-demographic and pregnancy outcome variables.

Results: Obese pregnant women were significantly less educated, had more medically assisted conceptions, more stress in history and higher parity compared to normal weight women. Significant stress was present in 48.4% of obese, versus 25.8% of controls (p<0.001). The levels of state anxiety significantly increased from trimester 1 to trimester 3 in pregnant obese women (p=0.02), while this parameter remained constant throughout pregnancy in normal-weight women (p=0.01). Levels of trait anxiety and depressed mood significantly decreased from trimester 1 to trimester 2 in controls, but not in pregnant obese women (p<0.001). Maternal education, ethnicity, marital state, history of stress and miscarriages, parity and smoking behavior had significant effects on anxiety and/or depressed moods during pregnancy.

Conclusions: Pregnant obese women show higher levels of anxiety and depressive symptomatology compared to normal-weight women at the start of pregnancy. The reduction in anxiety and feelings of depression observed during pregnancy in normal weight women is not seen in obese mothers.

F-255

Socio-Demographic Correlates of Pre-Pregnancy Body Mass Index and Gestational Weight Gain in the Region of Flanders, Belgium. Annick Bogaerts,[2] Bea Van den Bergh,[3] Erik Nuyts,[2] Evelyne Martens,[4] Ingrid Witters,[5] Guy Martens,[4] Roland Devlieger.[1] *[1]Department of Obstetrics and Gynaecology, University Hospitals KULeuven, Leuven, Belgium; [2]PHL University College, Dpt.PHL- Healthcare Research, KHlim, Limburg Catholic University College, Hasselt, Belgium; [3]Department of Psychology, Tilburg University, Tilburg, Netherlands; [4]Flemish Centre for the Study of Perinatal Epidemiology, Flemish Government, Brussels, Belgium; [5]Department of Prenatal Diagnosis, ZOL Ziekenhuis Oost-Limburg, Genk, Belgium.*

Background: No data on prepregnancy body mass index (BMI) and gestational weight gain (GWG) are available for the region of Flanders, Belgium. ***Objective***: We aimed to study the prevalence and distribution of prepregnancy BMI and GWG for the region of Flanders related to socio-demographic characteristics

Methods: Data of 54 022 singleton term pregnancies from the SPE registry in 2009 were analysed and linked to data from 'Vlaams Agentschap Zorg & Gezondheid'. BMI and GWG were categorized in accordance to the IOM. Adjusted associations of prepregnancy BMI and GWG with socio-

Friday

demographics, parity and pregnancy outcomes were estimated by linear and logistic regression analyses using the SAS program. ***Results***: In 2009 in Flanders, 21.7% of pregnant women were overweight and 10.2% obese. Variables associated with high prepregnancy BMI were low maternal education, maternal age and parity, Moroccan and Turkish ethnicity, low social state and presence of partner. In total, 38.5% gained within IOM recommendations while 58% of overweight and 54% obese had excessive GWG. Variables associated with excessive GWG were low prepregnancy BMI, primiparity, prolonged pregnancy, hypertension, Turkish ethnicity, level of maternal education, no diabetes in pregnancy, decreasing maternal age and level of employment. Overweight and obese pregnant women demonstrated higher odds of having excessive GWG, while underweight pregnant women report higher odds of inadequate GWG compared to the normal weight women. Women aged 20 – 24 years and singles are vulnerable for excessive as well as for inadequate GWG compared to the reference group, aged 25 – 29 years old and women cohabiting with a partner resp. ***Conclusions***: Both maternal overweight and obesity as well as excessive GWG are common in the studied region. Older, less educated multiparous women and women of Turkish and Moroccan origin are at the highest risk of being overweight or obese prior to pregnancy.

F-256

Gestational Weight Gain Prior to Glucola and Risk of Gestational Diabetes Mellitus. Katherine Callaghan, Anna BuAbbud, Xun Liao, Tiffany A Moore Simas. *Obstetrics and Gynecology, University of Massachusetts Medical School, Worcester, MA, USA.*

Background: Gestational diabetes mellitus (GDM) complicates 4–7% of U.S. pregnancies. Latinas are at risk, as diabetes and obesity rates are consistently higher in Hispanics compared to non-Hispanic whites. Though early-to-mid gestational weight gain (GWG) has been thought to be associated with GDM risk, the Institute of Medicine (IOM) found insufficient evidence when re-examining GWG guidelines in 2009. Our objective is to investigate GWG adherence as per 2009 IOM guidelines prior to 1-hour 50g glucola test and associations with GDM diagnoses in Latinas.

Methods: A retrospective chart review is being conducted of all Hispanic women delivered by UMassMemorial faculty between 4/1/06-3/31/11 who received prenatal care at faculty or resident practices (n=1226). Pre-pregnancy weight and height, weight and gestational age (GA) most proximate to glucola and 100g Glucose Tolerance Test (GTT) where appropriate, lab results and relevant demographics will be abstracted. Weight gain is categorized as inadequate, appropriate or excessive according to 2009 IOM Guidelines with adjustment for GA.

Results: At time of writing, data for 135 subjects has been analyzed. Preliminary cohort was mean age 26.1 years (sd±6.8), mean gravidity 3.1 (sd±2.2) and 71.1% English and 28.2% Spanish-speaking. Two subjects excluded for pregestational diabetes. BMI calculable for 109 subjects (2.8% underweight, 45.9% normal, 28.4% overweight and 22.9% obese); 36 subjects missing GWG prior glucola. Ninety-nine subjects have complete data: 18 of 99 diagnosed with GDM (18.2%). By 2009 IOM guidelines, 3 of 29 (10.3%), 7 of 20 (35.0%) and 8 of 50 (16.0%) with inadequate, appropriate and excessive gain respectively were diagnosed with GDM. No significant association has been found between pre-glucola GWG and GDM (p=0.08).

Conclusion: The rate of GDM in this preliminary cohort of Latina women is more than double that of the general population. Though no statistically significant association has been identified, fewer undergainers and overgainers were diagnosed with GDM compared to appropriate gainers in the current population. Further evaluation is needed as this study is completed.

F-257

Evaluation of the Frequency of Consumption of Foods Containing Genotoxic Compounds in a Cohort of Mexican Pregnant Women. Marisol Castillo-Castrejon,[1] Alejandra Migoya,[1] Noemi Meraz-Cruz,[1] Adriana Castanon,[1] Marie S O'Neill,[2] Felipe Vadillo-Ortega.[1] *[1]Department of Biochemistry, School of Medicine, Universidad Nacional Autonoma de Mexico, Mexico; [2]Department of Environmental Health, School of Public Health, University of Michigan, Michigan, USA.*

Background and Aims

Prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) are associated with increased risk for adverse birth outcomes. In addition to air pollution, foods may contribute to PAH/HCA exposures, which form depending on cooking methods. To evaluate the contribution of ambient pollution exposures to pregnancy outcomes, we are following a cohort of low-socioeconomic status pregnant women in Mexico City. The aim of this preliminary study was to assess the frequency of dietary PAH/HCA intake and consumption habits in our cohort.

Methods

A food frequency test and a 24-hour dietary recall were applied to participants at different times during prenatal follow-up. This questionnaire includes foods with a high content of PAHs/HCAs. Additionally, we are collecting information on participants' air pollutant exposure, medical, obstetrical and nutritional variables.

Results

Data from 300 women were analyzed. Total energy intake was 1954 ± 421.9 (mean±SD) calories per day. Dietary distribution of macronutrients was 15.4% proteins, 54.7% carbohydrates and 29.6% lipids. Homemade breaded fried chicken, deep-fried tacos and cooked pork skin were an important source of PAHs/HCAs. Other foods such as grilled tomato sauce, toasted bread, grilled cactus and toasted tortilla were also consumed on daily basis. Women consumed more dietary PAH/HCAs from cooked cereals and vegetables than from meat sources.

Conclusions

Main foods contributing to PAH/HCA intake in a Mexican urban pregnant population were identified. This pattern of consumption reflects the frequent intake of low-cost homemade foods with high PAH/HCA content. No quantitative studies of PAHs/HCAs are available for Mexican foods. Future plans include determining the relative contributions of dietary PAHs/HCAs, air pollution, and tobacco smoke exposures to DNA damage during pregnancy by correlating these exposures to PAH/HCA DNA adducts in mother's and umbilical cord blood. We will also evaluate associations between DNA damage and pregnancy outcomes.

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Compliance with Postpartum Glucose Screening in Patients with Gestational Diabetes Mellitus. Michael Demishev,[1] Terrissa Martin,[2] Wendy Kinzler,[3] Martin Chavez,[3] Anthony Vintzileos.[3] *[1]Obstetrics and Gynecology, Stony Brook-Winthrop University Hospitals, New York, NY, USA; [2]Obstetrics and Gynecology, New York University Hospital, New York, NY, USA; [3]Obstetrics and Gynecology, Winthrop University Hospital, Mineola, NY, USA.*

OBJECTIVE: To determine the frequency of patients with gestational diabetes (GDM) who receive the recommended postpartum glucose screening and to identify factors that are associated with suboptimal follow-up.

STUDY DESIGN: This was a retrospective cohort study of women with GDM identified through our perinatal database from January 2008 to December 2010. Patients less than 18 years old or with pre-gestational diabetes were excluded. Subjects not receiving a 75 g GTT within 12 weeks of delivery were considered suboptimally screened in accordance to ACOG and ADA guidelines. Office records were reviewed for demographic, antenatal, and postpartum factors. Univariate analyses were performed using $\chi 2$ and t-test. For independent variables with $P < 0.05$ multivariate logistic regression was applied to identify factors associated with suboptimal screening.

RESULTS: 152 patients met inclusion criteria; 92 (60%) were suboptimally screened. Of the 92 (60%) who did not receive screening, 12 (13%) had no post-partum follow-up visit. In 49 (35%) of 140 cases who had postpartum follow-up, there was no documentation regarding GDM diagnosis in the postpartum note. The following variables had $P < 0.05$ in univariate analyses: test recommended in the note, GDM noted in the history, postpartum follow-up, missed prenatal appointments, health care provider, mode of delivery, and delivery complications. Multivariate analyses indicated that suboptimal screening was more likely with one or more missed prenatal appointments (OR 8.6 (2.8, 26.6)) and less likely when recommendations were documented in the postpartum note by the health care provider (OR 0.1(0.0, 0.2)).

CONCLUSION: The frequency of post-partum glucose screening in women with GDM is suboptimal. More than half (60%) of women with GDM are not receiving the recommended postpartum follow-up. Implementation of systems based practices should be employed to correct factors that prevent optimal screening at both the physician and patient levels.

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The Effect of Isotonic Drinks during Labour on Pregnancy Outcome: A Randomized Controlled Trial. Nathalie Ciura, Marc Vandevelde, Helene Offeciers, Greet Jorissen, Cathrien Corthout, Roland Devlieger. *Department of Obstetrics and Gynaecology, University Hospitals KULeuven, Leuven, Belgium.*

OBJECTIVES: Labour is a period of high fluid and energy expenditure. We wanted to determine whether administration of isotonic drinks to pregnant woman during labour improves the outcome for mother and child.

METHODS: Prospective, randomized, controlled trial at the delivery unit of the University Hospitals Leuven, Belgium. Patients (N=119) presenting in early labour (cervical dilatation < 6cm) with singleton pregnancies between 37 and 42 weeks gestation during the study period were randomized to receive either isotonic sport drinks (N=59) or water (N=59) during labour. Both groups were allowed additional oral food intake. Calorific intake and outcomes in relation to labour, birth and comfort were recorded using a quantitative questionnaire and assessments of the patients' record. Maternal glycemia, ketonuria and neonatal pH were measured at birth.

RESULTS: Baseline characteristics (Age, BMI, Parity, GA at delivery, gestational weight gain, socio-demographics) were comparable between both groups. The liquid caloric intake was significantly higher in the study group (293+/-153 vs 165+/- 128; p<0.001) but the mean total caloric intake was comparable between both groups (804+/-566 vs 781+/-679; P=0.8). Duration of labour (7.3+/-3.6 vs 7.1+/-4.1; p=0.74) and other obstetric- and clinical outcomes of mother and newborn were not significantly different between the groups. There was a trend towards more CTG abnormalities in the control group (P=0.08). Maternal glycemia (128.3+/-39.7 vs. 130.7+/-45.7; p=0.78) was comparable. Less women showed ketonuria in the study group, but this difference was not significant (14% vs 27%: p= 0.32). More women reported hunger in the control group (p=0.05). More women in the study group experienced nausea and vomiting , but these differences were not significant.

CONCLUSION: Isotonic sport drinks provide a safe and well tolerated alternative to water for women in labour and are associated with higher liquid caloric intake and reduced feeling of hunger during labour.

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Changes in Leptin Concentration in Gestational Diabetes vs. Non-Diabetic Controls: A Longitudinal Assessment Antepartum and Postpartum. Rebecca I Epstein,[1] Christy F Pearce,[1] Samantha Mast,[1] Kevin Pearson,[2] Karen S Playforth,[1] John M O'Brien.[1] *[1]Obstetrics and Gynecology, University of Kentucky, Lexington, KY, USA; [2]Nutritional Sciences, University of Kentucky, Lexington, KY, USA.*

Background: Leptin is produced by adipocytes, primarily in visceral fat. Levels are elevated in obesity leading to leptin insensitivity which is involved in the metabolic syndrome. Concentrations are elevated in normal pregnancy, peaking in midgestation. Longitudinal data of leptin concentrations is limited, however, particularly in women with GDM.

Objective: To evaluate leptin levels in GDM vs. non-diabetic controls in pregnancy and postpartum and correlate them with BMI and weight change.

Study Design: Pregnant women (17 GDM, 15 controls) were enrolled between 24 and 34 weeks gestation. Serum leptin levels were obtained at enrollment and 6 -12 weeks postpartum (13 GDM, 12 controls). Changes in leptin were correlated with BMI and changes in weight using *t*-test, ANOVA, and linear regression analysis.

Results: Twenty-five of the 32 patients were defined as obese with BMI >30 (13 Class I, 7 Class II, and 5 Class III). During pregnancy, higher leptin levels were seen with increasing BMI (p <.0001). Postpartum levels were not related to BMI (p= 0.163). However, a larger change in BMI was associated with a larger change in leptin levels (p= 0.0275). Furthermore, a larger postpartum weight loss correlated with a greater decline in leptin levels (p= 0. 0226). There was no correlation between GDM and leptin levels ante or postpartum. Weight loss was not significantly different between those with GDM vs. controls (8.04 vs. 4.27 kg, p=0.189). Women with elevated fasting glucose postpartum (n=5) had higher initial leptin levels (p=.0542), but no significant differences in postpartum leptin levels or in the percent change from pregnancy to postpartum were observed in this subgroup, but the sample size is limited.

Conclusion: Elevated leptin levels were correlated with increasing BMI antepartum. We observed a larger magnitude of change in leptin concentration with greater weight loss postpartum, and BMI change. We did not identify a correlation between GDM and leptin levels in this cohort of mostly obese parturients. Leptin is increased in obese patients and may signify risk for persistent or long-term metabolic abnormalities. Further study is needed to assess this hormone's role and whether it can prognosticate the need for any alteration in clinical management.

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Pregnancy Cravings, Dietary Intake and Gestational Diabetes. Leslie V Farland,[1] Sheryl L Rifas-Shiman,[2] Matthew W Gillman.[2] *[1]Epidemiology, Harvard School of Public Health, Boston, MA, USA; [2]Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA.*

Background/Objective: While dietary cravings during pregnancy are common, their biological significance is not understood. Previous studies showed a relationship between gestational diabetes (GDM) and craving sweet foods in the third trimester of pregnancy. We examined prospective relationships of cravings in the first trimester with contemporaneous dietary intake and with risk of GDM or impaired glucose tolerance (IGT) later in pregnancy.

Methods: 2,044 mothers in Project Viva reported whether they had cravings at mean 10.9 weeks' gestation. We coded each craving as strong v. weak or none for each of 6 categories: sweet, salty, savory, starchy, non-sweet/dairy, other. The main outcomes were sucrose and glycemic load intake from a food frequency questionnaire at mean 11.8 weeks' gestation, and incident GDM or IGT determined by glucose tolerance screening at 26-28 weeks. We used linear and logistic regression adjusted for maternal age, race/ethnicity, education, smoking, pre-pregnancy BMI, and (for risk of GDM or IGT) GDM in a previous pregnancy.

Results: Mean (SD) age was 31.8 (5.2) years and pre-pregnancy BMI was 24.9 (5.5) kg/m2. 1191 (67%) women were white. In the first trimester 913 (44.7%) women reported any craving; 444 (21.7%) craved sweets, 225 (11.0%) craved salty foods, and 100 (4.9%) craved starchy foods. Mean (SD) for sucrose intake was 49.5 (15.5) gm/day and for glycemic load was 14,766 (2,273). 181 (9.7%) women developed GDM or IGT. In adjusted analysis, craving sweet food was associated with slightly higher intake of sucrose (1.6 gm/day; 95% CI -0.2, 3.3), and craving starchy food was associated with higher glycemic load (501; 95% CI 17, 985). Craving sweet or starchy food was not associated with GDM or IGT (OR 1.21; 95% CI 0.80, 1.83, and OR 0.72; 95% CI 0.27, 1.91, respectively). However, craving salty foods was associated with reduced odds of GDM or IGT (OR 0.45; 95% CI 0.21, 0.95).

Conclusions: Cravings for starchy foods in the first trimester was associated with higher contemporaneous glycemic load. Craving sweet or starchy foods in early pregnancy was not associated with later diagnosis of GDM or IGT. Craving salty foods during early pregnancy may be a marker of reduced risk for developing GDM or IGT.

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Body Mass Index as a Predictor of Failed Trial of Labor among Extremely Obese Women. Ravindu Gunatilake,[1] Michael Smrtka,[1] Benjamin Harris,[2] Daniel Kraus,[1] Chad Grotegut,[1] Maria Small,[1] Haywood Brown.[1] *[1]Obstetrics and Gynecology, Duke University, Durham, NC, USA; [2]School of Medicine, University of North Carolina, Chapel Hill, NC, USA.*

OBJECTIVE: Obesity is an independent predictor of cesarean delivery (CD). The purpose of this study was to examine predictors associated with vaginal delivery (VD) among extremely obese patients undergoing a trial of labor (TOL).

STUDY DESIGN: Using a delivery database, we identified all pregnant patients delivering at our institution from January 1, 2008 through July 31, 2010 weighing greater than 275lbs (all BMI > 40) at the time of delivery. Among these women, we identified those with a singleton gestation, who were greater than 34 weeks' gestation, and undergoing a TOL. Descriptive statistics, Chi-Square tests, and multiple logistic regression (MLR) were utilized for analysis and p<0.05 was deemed significant.

RESULTS: We identified 357 obese subjects greater than 34 weeks' gestation carrying a singleton pregnancy, and among these, 262 (73.4%) attempted a TOL. Of those attempting a TOL, the median (quartile) BMI was 49.6 (46.7, 53.9) and the induction rate was 63%. The mean (SD) maternal age was 30.2 years (6.2), gestational age at delivery was 38.5 weeks (2.3), and cervical dilation on admission was 2.2 cm (2.1). The CD rate for the entire group (n=357), stratified by BMI category (40-50, >50-60, >60) was 55%, 65%, and 80% respectively (p<.005). Among those undergoing a TOL, VD occurred in 61%. The CD rates differed between primigravida and multigravida undergoing a TOL (55% vs 25% , p<.0001). All of the variables in the MLR model below

were independent predictors of successful VD with the exception of induction status (Table). An increase in BMI of 10 kg/m2 was associated with a 50% reduction in the odds of a successful VD.
CONCLUSION: Among extremely obese women attempting a TOL, BMI was an independent predictor of VD, with the rate of VD falling as BMI increased. The underlying mechanisms for labor dysfunction in the setting of maternal obesity remain largely unknown.

Extreme BMI at admission and predictors of vaginal delivery

	OR	95% CI	p-value
Maternal age (1)	0.9	(0.9, 1.0)	0.04
Multiparity	4.6	(2.4, 9.2)	<0.0001
Cervical dilation at admission (2)	1.6	(1.3, 2.0)	<0.0001
BMI (3)	0.5	(0.3, 0.8)	0.0003
Induction of labor	0.9	(0.4, 2.0)	0.8

OR for 1-year increase in age (1), 1-cm increase in cervical dilation (2), and 10 kg/m2 increase in BMI (3)

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Association with HbA1c in Maternal Blood and Lipid Peroxidation and Protein Oxidation in Umbilical Venous Plasma in Normal Pregnancy, Gestational, and Overt Diabetes Mellitus. Yoon Ha Kim, Hye Yon Cho, Jong Woon Kim, Tae-Bok Song. *Obstetrics & Gynecology, Chonnam National University Medical School, Gwangju, Korea.*

Objective: We would like to identified the relationship between the pathophysiology of diabetic pregnancy and fetal outcomes by comparing level of HbA1c in maternal blood and oxidative stress through levels of lipid peroxidation and protein oxidation in umbilical venous plasma, in normal pregnancy, gestational diabetes mellitus(DM), and overt DM within insulin therapy.

Materials and Methods: We obtained maternal venous blood for HbA1c and maternal capillary blood for fasting blood sugar (FBS) from 19 normal pregnant women, 12 gestational DM, and 10 overt DM within insulin therapy, all over 25 weeks of gestation. Umbilical venous plasma was taken during delivery from each groups and serum was acquired from cetrifuge. Lipid peroxide, and protein carbonyl levels were measured by thiobarbituric acid reaction, 2,4-dinitrophenyl hydrazine method, respectively.

Results:

1. Basal lipid peroxide levels in umbilical venous plasma were significantly higher in gestational DM group and overt DM group than normal pregnancy group ($p<0.001$. $p<0.05$). Also, basal protein carbonyl levels were significantly higher in gestational DM group and overt DM group than normal pregnancy group ($p<0.001$, $p<0.01$).
2. HbA1c levels in maternal venous blood before delivery were significantly higher in gestational DM group (6.45±0.51 %) and overt DM group (7.90±0.72 %) than normal pregnancy group (5.01±0.29 %) ($p<0.01$. $p<0.01$).
3. When we compared levels of HbA1c, more than 6% of HbA1c group (5.01±0.30 and 6.71±0.28 mmol/mg) had more higher levels of lipid peroxides and protein carbonyls than those of less than 6% of HbA1c group (2.98±0.10 and 4.99±0.10 mmol/mg) ($p<0.001$, $p<0.001$).
4. There was no significant difference in levels of lipid peroxides and protein carbonyls between FBS over 95 mg/dl group and less than 95 mg/dl group.

Conclusion: The increased lipid peroxide and protein carbonyl levels in umbilical venous plasma means the increment of oxidative stress in gestational DM and overt DM pregnant women. And, it might be associated with the increased HbA1c levels in maternal venous blood. Strongly, this suggests that the blood sugar control for a considerable period causes the decline of oxidative stress and then, might lead to the good fetal outcomes.

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Changes of Lipid Peroxide Levels, Protein Carbonyl Levels, and Antioxidative Ability in Maternal Venous Plasma after Red Ginseng Supplementation during Postpartum Period after Cesarean Section. Yoon Ha Kim, Jong Woon Kim, Hye Yon Cho, Tae-Bok Song, Jin Wook Kim. *Obstetrics & Gynecology, Chonnam National University Medical School, Gwangju, Korea.*

Objectives: The aim of this study was to investigate and compare the changes of the lipid peroxidation, protein carbonyls formation, and oxygen-radical absorbance capacity (ORAC) in maternal venous plasma after red ginseng supplementation during postpartum period after cesarean section.

Materials & Methods: Forty-one pregnant women who were scheduled for elective cesarean section were randomized in this study. Twenty women were given daily oral dose of red ginseng extracts 4,500 mg from postoperative day (POD) 4 to 28 (ginseng group). The other twenty-one women were placebo given, as a control group. Maternal venous blood were obtained from the two groups before the operation, and POD 3, POD 10 and POD 28. Lipid peroxide, protein carbonyl, and ORAC levels were measured by thiobarbituric acid reaction, 2,4-dinitrophenyl hydrazine method, and Cao's method, respectively. The differences between preoperation and POD3, POD3 and POD 10, and POD3 and POD 28 were compared.

Results: The protein carbonyl levels was significantly decreased between POD 3 and POD 28 in ginseng group (5.010±0.087 vs. 4.720±0.077 nmol/mg protein, $P<0.05$), whereas it did not show any difference in control group. The ORAC levels was significantly decreased P between OD 3 and POD 28 in control group (30063.00±162.449 vs. 29468.308±232.223 U/ml, $P<0.05$), whereas it did not show any difference in ginseng group. The lipid peroxide levels was significantly increased between preoperation and POD 3 in both groups (1.479±0.084 vs. 2.026±0.149 nmol/mg protein, $P<0.01$, 1.551±0.111 vs. 1.854±0.144 nmol/mg protein, $P<0.01$), and it was significantly decreased between POD 3 and POD 10 (2.026±0.149 vs. 1.057±0.088 nmol/mg protein, $P<0.01$, 1.854±0.144 vs. 0.975±0.104 nmol/mg protein, $P<0.01$), and POD 3 and POD 28 in both groups (2.026±0.149 vs. 0.866±0.065 nmol/mg protein, $P<0.01$, 1.854±0.144 vs. 0.851±0.078 nmol/mg protein, $P<0.01$).

Conclusion: Red ginseng supplementation decreases the protein carbonyl formation and prevents the reduction of antioxidative ability in maternal blood during the postpartum period after cesarean section. This results suggest that the red ginseng supplementation during the postpartum period may increase the antioxidative effect for a patient who had a cesarean section.

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Utilization of Triage Care by Women with Increased Body Mass Index. Mollie A McDonnold, Alissa Carver, Tony Wen, Gayle Olson. *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: Increased body mass index (BMI) is associated with increased morbidity, prenatal care, ultrasounds, and hospital length of stay. It is unclear whether these women utilize non-routine obstetric services greater than their non-obese counterparts. The objective of this study was to examine the influence of BMI on triage visits.

Study Design: A retrospective cohort review examining patients presenting to a tertiary care obstetric triage unit serving multiple regional clinics over a 3 month period in 2010. Records were individually reviewed. Visit indication and referral data was compared between obese women (BMI ≥30) and non-obese women. Statistical analysis was performed using t tests and Chi square.

Results: Of 1017 triage visits by 745 patients, 639 (62.8%) were unscheduled non-term labor (UNTL) related. Obese women represented 28.9% of the study population but 38.1% of UNTL visits. Differences between the two groups are displayed (Table 1). Obese women had an average of 1.12 UNTL compared to 0.74 for non-obese women ($p < 0.0001$). In addition, obese vs. non-obese women were more likely to require transfer from the outpatient to the triage setting (40.4% vs. 18.9%, $p<0.0001$), with an odds ratio of 2.9 (CI 2.06 – 4.14) of requiring transfer at least once during pregnancy. Indications for UNTL visits were more often associated with preeclampsia for obese women (16.8% obese visits vs. 8.5% non-obese, $p<0.0001$) and contractions at term for non-obese women (16.7% obese visits vs. 31.1% non-obese, $p<0.0001$). Differences between other visit indications (preterm labor, decreased fetal movement, vaginal bleeding, fetal evaluation) were not significant.

Table 1. Population Demographics

	BMI > 30 (n = 215)	BMI < 30 (n = 530)	P value
Age (mean), yrs	28.19 + 6.42	25.15 + 5.82	0.04
Gravidity (mean)	3.0 + 1.8	2.36 + 1.56	0.38
BMI (mean)	35.92 + 6.7	24.27 + 3.35	<0.001
Diabetes (%)	40 (18.1)	33 (5.3)	<0.001
Chronic Hypertension (%)	30 (13.6)	16 (3)	<0.001

Conclusion: Increased BMI is associated with increased triage utilization and an increased likelihood of transfer from clinic to a higher level of care. This may be influenced by an increased baseline incidence of comorbities, but nonetheless provides supportive evidence that obese women require increased costs and resources and supports the classification of obesity as an increased risk group in pregnancy.

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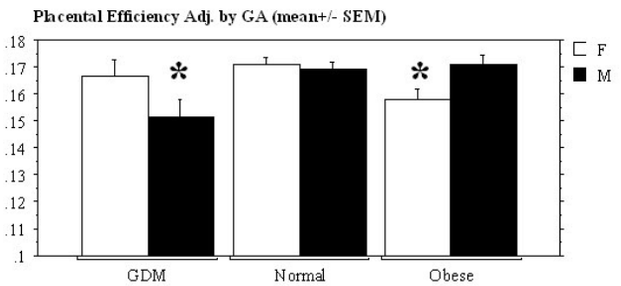
Maternal Obesity and Gestational Diabetes Are Associated with Reduced Placental Efficiency. Ellen Flatley, Amy Schilling, Terry Morgan. *Pathology and Obstetrics & Gynecology, Oregon Health & Science University.*

Background: Nearly one-third of reproductive-age women are obese and many of these pregnancies are complicated by gestational diabetes, which significantly increases the risk of stillbirth. The mechanisms underlying these increased risks are poorly understood. We hypothesize that obesity

and gestational diabetes may significantly increase relative placenta growth, thereby decreasing available nutrients to the fetus, so-called reduced placental efficiency.

Methods: Retrospective analysis of 518 singleton placentas with available maternal metrics, including pre-pregnancy body mass index (BMI), gestational diabetes diagnosed by glucose tolerance test, gestational age at delivery, birth weight, placental weight, and neonatal gender. Obesity was defined as BMI of 30kg/m2 or greater (n=171). Gestational diabetes cases were grouped together regardless of BMI (n=40). Placental efficiency was defined as birth weight (grams)/placental weight (grams) adjusted by gestational age. Data were analyzed by ANOVA with Bonferroni/Dunn post hoc testing.

Results: Placental efficiency increases with gestational age (R=0.58, p<0.0001) as fetal weight increases faster than placental weight. Therefore, all analyses were performed after adjusting for gestational age. We observed significant gender-effects. Compared to mothers with normal BMI (n=307), female babies from obese women (n=81) had significantly less placental efficiency (P<0.05) due to markedly increased placental weights (P<0.01). Males (n=18) showed less placental efficiency only in the presence of gestational diabetes (P=0.05), again due to increased placental weight (P<0.01). There was a trend towards increased birth weight related to gestational diabetes, but unlike placenta weight, they were not statistically significantly.



Conclusions: Given the current obesity epidemic, a better understanding of the relationship between maternal BMI, gestational diabetes, and placental efficiency is required. Our data reveal significant gender effects and the potential importance of reduced placental efficiency in complications associated with maternal BMI and gestational diabetes.

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Maternal Nutrient Restriction Down Regulates Cardiac Mitochondrial Proliferation in Male Non-Human Primates (NHP) at 0.5 Gestation (G). Susana P Pereira,[1,2] Paulo J Oliveira,[1] Laura A Cox,[3] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Center for Neurosciences and Cell Biology, Univ. Coimbra, Coimbra, Portugal; [2]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA; [3]Department of Genetics, TexBiomed, San Antonio, TX, USA.*

Objective : Nutrition and other environmental stimuli influence developmental pathways, inducing permanent changes in metabolism and chronic disease susceptibility. How early environmental stress induces persistent molecular changes is not well understood. Cell specific ablation of mitochondrial genes in experimental models has been linked to cardiac dysfunction and diabetes. More importantly, abnormal mitochondrial function is associated with obesity, diabetes and hypertension.

Method : We performed transcriptome analysis (TA) using Illumina Bead-Chip arrays and male NHP fetuses of *ad lib* fed controls (C) and dams 70% of C (MNR; adjusted to body weight) from 0.16G to tissue collection at 0.5G. Samples were taken from the free wall of the cardiac left ventricle.

Results : TA revealed 1786 significantly differentially expressed genes, with 968 up-regulated and 818 down-regulated by MNR. Of these, 41 genes play roles in mitochondrial respiratory chain/metabolism (10 up, 31 down-regulated), including cytochrome b5 type B (CYB5B; 126% down) and cardiolipin synthase 1 (CRLS1; 18% down), and 30 genes involved in mitochondrial genetics and protein transport (1 up, 29 down-regulated), including mitochondrial transcription factor A (TFAM; down 28%), mitochondrial translation initiation factor 2 (MTIF2; down 22%) and 16 genes encoding ribosomal proteins (down 11-30%).

Conclusions : A large number of mitochondrial transcripts are down regulated by MNR at 0.5 G in male cardiac left ventricle, including many components of complex I and complex V, mitochondrial ribosomes and mitochondrial protein import and biogenesis. Importantly, the expression of CRLS1, which participates in the synthesis of cardiolipin, a co-factor for many mitochondrial enzymes including the adenine nucleotide translocator and complex IV (cytochrome c oxidase), is also decreased. These findings suggest that MNR from 0.16 to 0.5 G suppresses mitochondrial proliferation leading to decreased mitochondrial mass in cardiac left ventricle of the male NHP fetus.

Supported by NIH PO1 HD023150 (MJN, PWN) and the Portuguese Foundation for Science and Technology SFRH/BD/64247/2009 (SPP,PJO).

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Twizzlers as a Cost Effective and a Equivalent Alternative to the Glucola Beverage in Screening for Gestational Diabetes (GDM). Diana Racusin,[1] Sara Andrabi,[1] Nelli Crawford,[1] Haleh Sangi-Haghpeykar,[1] Lori Showalter,[1] Susan Sharma,[2] Morey Haymond,[2] Kjersti Aagaard.[1] *[1]Obstetrics & Gynecology, Baylor College of Medicine; [2]Pediatrics, Baylor College of Medicine.*

OBJECTIVE: GDM screening employs a glucose challenge test (GCT) with a 50g glucola beverage followed by venipuncture. Up to 30% report side effects and the beverage is costly ($3.25-7.80). Alternatives have not been widely validated. We hypothesized that equivalent glucose loads could be calculated from candy and tested as alternatives. We evaluate Twizzlers as an alternative with a test of equivalency.

STUDY DESIGN: Employing standard mean estimates (90 +/- 10g/dL), a sample of n=20 was calculated in a triple crossover study (80% power to detect equivalence at a margin of 7 [-7,7] and actual difference of 0). For GCT, subjects consumed Twizzlers (week 1), then 50g glucola (week 2) over <5min. 1hr serum glucose (g/dL), lactate (mmol/L), and insulin (uU/ml) levels were determined by clinical chemistry and immunoassay. Screen positive was per accepted values of ≥130 or 140 mg/dl, and a diagnostic 3hr GTT was performed in week 3 on all subjects with diagnostic criterion as per Coustan or National Diabetes Data Group (NDDG). Significance was inferred by paired t-tests and variance of means and quartiles (SPSS and SAS).

RESULTS: At either ≥130 or 140mg/dl, an equivalent subject ratio (6.8%) "screened positive" with Twizzlers or 50gm glucola (paired t-test p=.78; r=0.45, p=0.04); this did not vary by BMI.

Table 1

Maternal BMI	**Glucola Beverage Serum Glucose (Mean±SD, g/dl)**	**Twizzler Serum Glucose (Mean±SD, g/dl)**	***p***
Overall (n=20)	105.91±65.34	108.59±36.78	0.78
<25 kg/m2 (n=16)	99.83±36.26	102.56±30.82	0.88
≥25 kg/m2 (n=4)	130.23±66.04	132.75±53.33	0.82
	Serum Insulin (mean±SD, uU/ ml)	**Serum Insulin (mean±SD, uU/ ml)**	
Overall (n=20)	42.28±26.39	33.65±26.58	0.10
<25 kg/m2 (n=16)	39.57±20.56	27.17±11.73	**0.035**
≥25 kg/m2 (n=4)	53.09±45.86	59.55±51.66	0.61

1 participant met Coustan diagnostic criteria and none by NDDG; this subject screened positive by Twizzlers and glucola. At >139mg/dl, equivalent sensitivity (100%), specificity (89.5%), PPV (33.3%), and NPV (100%) were observed. At >129mg/dl, sensitivity and NPV remained unchanged but specificity (78.95% vs 84.2%) and PPV (20% vs 25%) minimally varied. A lower serum insulin in the overall and <25 kg/m2 BMI cohorts was observed with Twizzlers (p=0.035).

CONCLUSION: Twizzlers provide an eqivalent and cost effective alternative to the glucola beverage for GCT.

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Placental Insulin Resistance in Women with High BMI: Insulin Receptor Expression and Insulin Signaling. Vanessa I Ramirez, Francesca Gaccioli, Thomas Jansson, Theresa L Powell. *Center for Pregnancy and Newborn Research, Dept OB/GYN, Univ of Texas Health Science Ctr, San Antonio, TX, USA.*

Introduction: Pregnant women with high BMI have an increased risk to develop gestational diabetes and to give birth to a large baby. Overweight and obese pregnant women are typically more insulin resistant and have higher fasting levels of insulin, as compared to lean women. We hypothesized that women with high pre-pregnant BMI have altered placental insulin receptor expression and activation of placental insulin signaling. **Methods:** Paired maternal and umbilical fasting blood samples (n=21) were obtained at cesarean delivery from predominantly Hispanic women with normal BMI (23.0 ± 0.9; n=8) and high BMI (33.6 ±1.9; n=13). Serum insulin levels were analyzed by ELISA. Placentas were collected from women with normal BMI (22.2 ± 0.5; n=9) and high BMI (33.8±1.1; n=18) and syncytiotrophoblast microvillous plasma membranes (MVM) and basal plasma membrane (BM) were isolated. Insulin receptor expression (IR) was measured in MVM and BM by Western blot. Expression of phospho-Akt, phospho-glycogen synthase kinase-3 (GSK3), and phospho-insulin receptor substrate 1 (IRS-1), indicators of insulin signaling, was determined in placental homogenates by Western blot. **Results:**

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Birth weights in high BMI women were increased (3653±64g) compared to normal BMI women (3469±62g). Maternal serum fasting insulin was 48% ($p<0.05$) higher in women with high BMI (12.07±1.33 μIU/ml) as compared to women with normal BMI (8.13±0.54 μIU/ml). In contrast, fetal serum insulin levels did not differ between the two BMI groups. The expression of the insulin receptor in MVM was 3.6 fold higher ($p<0.01$) compared to BM of term placenta. MVM insulin receptor levels were 63% higher ($p<0.05$) in pregnancies complicated by high BMI compared to normal BMI pregnancies. BM expression of insulin receptor was not different between normal BMI and high BMI groups. Phosphorylation of placental IRS-1 (Ser 318), GSK3 (Ser 21/9), or Akt (Thr 308) was not significantly influenced by maternal BMI. **Conclusion:** High BMI in pregnancy is associated with elevated maternal fasting insulin and increased insulin receptor expression in the maternal-facing plasma membrane of the syncytiotrophoblast. However, there was no evidence of activation of placental insulin dependent pathways. These preliminary data suggest that the placenta, like the mother, is insulin resistant in pregnancies complicated by high maternal BMI.

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Maternal and Neonatal Outcome after Laparoscopic Adjustable Gastric Banding (LAGB): A Systematic Review Vrebosch Lore,[2] Sarah Bel,[1] Galjaard Sander,[1] Devlieger Roland.[1] *[1]Department of Obstetrics and Gynaecology, University Hospitals KULeuven, Leuven, Belgium; [2]Faculty of Medecine, Nursing and Midwifery, KULeuven, Leuven, Belgium.*

AIM

Concerns arise with regard to the potential impact of bariatric surgery on pregnancy. The objective of this study was to list both maternal and neonatal outcomes in pregnancy following LAGB .

METHODS

Identification of relevant studies was performed by a thorough search in databases Medline, DARE and NGC. Search terms included "pregnancy," "laparoscopic gastric banding," "bariatric surgery," "obesity," "neonatal/ maternal outcome" and "pregnancy outcome." Subsequently, the reference list of each relevant publication was screened for other relevant references. Maternal and neonatal outcomes were abstracted and presented in separate data tables. A discrimination between studies with or without a control group was made.

RESULTS

Ten studies were included: four observational studies with and six without a control group. We found a lower incidence of Gestational Diabetes Mellitus (GDM), pregnancy-induced hypertension (PIH), pre-eclamspia, caesarean section (CS), macrosomia and low birth weight babies in post-LAGB pregnancies than in non-LAGB pregnancies in obese women. All four studies showed a lower gestational weight gain (GWG) in post-LAGB pregnancies. However, the rate of spontaneous abortion and neonatal intensive care unit (NICU) admission was higher in post-LAGB pregnancies than non-LAGB pregnancies in obese woman. When comparing the outcomes of post-LAGB-pregnancies to the outcomes of pregnancies in normal weight women, the GWG post-LAGB also seems to be lower. However, the rate of adverse maternal and neonatal outcomes (PIH, pre-eclampsia, CS, preterm birth, large for gestational age (LGA), spontaneous abortion and NICU admission) seems to be higher in LAGB-pregnancies than in pregnancies of normal weight women. Band-related complications are possible during pregnancy after LAGB, but are not common. With active management of the gastric band, thus with anticipating problems, problems can be avoided.

DISCUSSION

Observational studies (case control and cohort studies) are the best available evidence. Furthermore, literature regarding long-term outcomes in children born to women with a gastric band or other bariatric surgery is not available.LAGB seems to be a useful and applicable way for improving pregnancy outcomes in (morbidly) obese women of reproductive age but implies new increased risks esp. band complications.

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The Effect of Glucose Variability on Neonatal Birth Weight. Rachelle A Schwartz, Barak Rosenn. *Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, New York, NY, USA.*

Objective: Poor glycemic control of pregnant women with diabetes is associated with an increased risk of diabetic fetopathy. Excessive glucose variability is considered a measure of poor glycemic control. The purpose of this study was to test the hypothesis that glucose variability is associated with neonatal birth weight in women with gestational diabetes (GDM).

Study Design: Women with GDM, delivery at ≥ 37 weeks gestation, documented birth weight, and complete blood glucose data during 2007-2010 were included. The coefficient of variation (CoV) was calculated for each patient's overall, fasting, and 2 hour post prandial glucose concentrations. Macrosomia was defined as ≥4000g, while large for gestational age (LGA) and small for gestational age (SGA) were defined as ≥90% and ≤10% for the given gestational age at birth, respectively. Statistical analysis was performed using Students t-test and linear regression.

Results: 351 subjects were included. The overall incidences of macrosomia, LGA, and SGA were 4.3%, 9.7%, and 6%, respectively. The table shows the comparison of the CoV between macrosomic, LGA, and SGA neonates and their non-affected counterparts. There was no significant correlation between birth weight or birth weight percentile and each individual CoV category (Pearsons correlation coefficients range:-0.03 to -0.05).

Conclusions: Among neonates born to diabetic mothers, aberrations of growth (macrosomia, LGA, or SGA) are not associated with an increase in glucose variability. Furthermore, there was no correlation between glucose variability and birth weight or birth weight percentile.

Table 1. Comparison of CoV between Abberant and Normal Fetal Growth Patterns

	Macrosomia (n=15)	No Macrosomia (n=336)	p
CoV Overall	19.9 (4.0)	20.1 (4.8)	0.87
CoV Fasting	12.8 (4.1)	13.9 (7.6)	0.56
CoV PP	17.5 (4.2)	18.3 (6.4)	0.63
	LGA (n=34)	No LGA (n=317)	p
CoV Overall	20.8 (4.8)	20.5 (4.8)	0.73
CoV Fasting	13.6 (5.4)	13.9 (7.6)	0.74
CoV PP	18.2 (5.2)	18.3 (6.4)	0.93
	SGA (n=32)	No SGA (n=319)	p
CoV Overall	19.9 (3.2)	20.6 (4.9)	0.41
CoV Fasting	12.8 (5.5)	13.6 (5.8)	0.42
CoV PP	18.5 (5.8)	18.3 (6.3)	0.92

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Diabetes-Associated Genetic Variants and Gestational Weight Gain among African-American Women. Dana Smith,[1] Amy Herring,[2,3] Alison Wise,[2] Anna Maria Siega-Riz,[3,4,5] Alison Stuebe.[1] *[1]Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, NC, USA; [2]Department of Biostatistics, Gillings School of Global Public Health, Chapel Hill, NC, USA; [3]Carolina Population Center, University of North Carolina, Chapel Hill, NC, USA; [4]Department of Epidemiology, Gillings School of Global Public Health, Chapel Hill, NC, USA; [5]Department of Nutrition, Gillings School of Global Public Health, Chapel Hill, NC, USA.*

Objective: Weight gain is a known risk factor for diabetes. Recent genome-wide association studies have identified single nucleotide polymorphisms associated with type 2 diabetes risk. We sought to determine the association between diabetes risk allele carriage and gestational weight gain among African American women.

Methods: We used data from the Pregnancy, Infection, and Nutrition Study, 1998-2005. Participants of self-reported African-American race from whom extracted DNA samples were available were genotyped for 20 single-nucleotide polymorphisms associated with diabetes in genome-wide association studies (n=397). Logistic regression was performed to model the association between genotype and gestational weight gain. All models were adjusted for maternal age, pregravid BMI, pregravid BMI squared, gestational age, gestational age squared, and probability of Yoruban ancestry. P values <0.05 were considered statistically significant.

Results: Mean total gestational weight gain was 13.5 kg (SD 7.7). Women with 1 or 2 copies of the G6PC2 (rs560887) risk allele gained 2.28 kg (96% CI 0.25, 4.31) more weight than women who were homozygous for the low risk variant. Conversely, homozygosity for the GCK (rs4607517) and PRC1 (rs8042680) risk allele carriage was associated with lower weight gain. Participants homozygous for these risk alleles gained -2.46 kg (95% CI -0.072, -4.84) and -2.35 kg (95% CI -0.47, -4.23), respectively, compared with heterozygotes or low risk homozygotes.

Conclusion: In a cohort of African-American women, we found that three diabetes-associated risk alleles were associated with differences in gestational weight gain. The G6PC2 and GCK risk alleles are associated with impaired fasting glucose in other studies. The PRC1 gene is involved in cytokinesis. These results suggest that genetic differences in glucose homeostasis may be associated with gestational weight gain.

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Fetal High-Density Lipoprotein (HDL) in Gestational Diabetes Mellitus (GDM) Moves to an Inflammatory Protein-Profile. Ivana Sreckovic,[1] Ruth Birner-Gruenberger,[2] Sonia Philipose,[3] Michael Holzer,[3] Monika Scholler,[4] Gunther Marsche,[3] Gernot Desoye,[1] Uwe Lang,[1] Christian Wadsack.[1] *[1]Clinic of Obstetrics and Gynecology, Medical University of Graz; [2]Proteomics Core Facility, Medical University of Graz; [3]Institute of Experimental and Clinical Pharmacology, Medical University of Graz; [4]Institute of Pathology, Medical University of Graz.*

OBJECTIVES: GDM is related to postnatal fetal obesity and to higher risk for vascular events. We hypothesized that qualitative, in addition to quantitative differences in HDL, which exhibits antioxidative properties, might be one of the determining factors responsible for risk in diabetes.

METHODS: HDL was isolated by Dynabeads-ProteinG from control and GDM maternal/fetal donors (n=11) and shotgun-proteomics was used for identification of proteins. Paraoxonase (PON-1) activity, lecithin:cholesterol-acyltransferase (LCAT), cholesterolester-transfer protein (CETP) mass and activity were quantified by kits. [^{3}H]-C efflux from arterial (HPAEC) and venous (HPVEC) endothelial cells to different acceptors was measured.

RESULTS: 44 distinct maternal/fetal HDL-associated proteins were detected. Healthy, maternal HDL was enriched in PON-1 ($p<0.01$) apolipoproteins (apoAI, apoCIV, apoF, apoL) ($p<0.05$), whereas control, fetal HDL was composed of a higher apoE rate ($p<0.05$). The same maternal/fetal differences were observed in GDM group, whit exception of PON-1, the most potent antioxidant protein. The lower PON-1 protein expression ($p<0,001$) was corroborated by lower PON-1 activity ($p<0.01$). HDL isolated from GDM mothers and fetuses was also deficient in apolipoproteins (apoCII, apoCIII, apoD, apoM) ($p<0.01$), coagulation proteins (prothrombin, pro-platelet protein) ($p<0.001$) and α1-antytripsin ($p<0.01$). Acute phase response proteins (serum amyloid A and complement components) dominated in both, maternal and fetal, GDM-HDL proteome ($p<0.05$). LCAT mass, CETP mass and activity were decreased in GDM plasma ($p<0.01$). Since quantity of apoE was almost equal and since apoE is the major activator for LCAT, the similar LCAT-activity in all groups appeared comprehensible. Control, fetal HDL showed higher efflux capacity of [^{3}H]-C from HPVEC ($p<0.01$), whereas in HPAEC was no difference among acceptors.

CONCLUSION: Fetal HDL is unique in respect to its concentration and composition. Altered GDM HDL proteome in both circulations is directly linked to functional consequences in lipid metabolism and inflammation.

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The Hemoglobin A1c Level and Adverse Pregnancy Outcome in Diabetic Gravidas. Roman S Starikov,[1] Donald R Coustan,[1] Edward KS Chien,[1] Michael Paglia,[1] Vrishali Lopes.[2] *[1]Obstetrics and Gynecology, Division of MFM, Women & Infants Hospital of RI and the Warren Alpert Medical School of Brown University, Providence, RI, USA; [2]Department of Research, Division of Statistics, Women & Infants Hospital of RI and the Warren Alpert Medical School of Brown University, Providence, RI, USA.*

Objective: To examine the association of hemoglobin A1c level and the development of adverse pregnancy outcome in women with preexisting diabetes mellitus in pregnancy.

Study Design: Retrospective cohort study. Women were identified at Women and Infants Hospital of Rhode Island who were in the Diabetes in Pregnancy Program between 2005 and present. Inclusion criteria were a singleton gestation, preexisting diabetes mellitus and baseline hemoglobin A1c (Hg A1c) recorded. The rates of adverse pregnancy outcome (defined as birth <37 weeks, preeclampsia, IUGR, IUFD), average age of delivery, shoulder dystocia, primary cesarean delivery and low APGAR scores (< 7 at 5 minutes) were compared between patients whose Hg A1c< 8.5% and patients whose HgA1c level ≥8.5%.

Results: Of 295 pregnancies, 104 patients (35.2%), had HgbA1c level ≥8.5. Mean gestational age at the time of Hg A1c was 9.8 weeks in patients with Hg A1c<8.5% and 8.4 weeks in patients with Hg A1c ≥8.5% (P<0.01). Patients with Hg A1c ≥8.5% were more likely to deliver preterm than patients with Hg A1c <8.5% (37.0 weeks vs. 35.8 weeks, P<0.005). Patients with HgA1c ≥8.5% were at higher risk for shoulder dystocia compared to patients with Hgb A1c <8.5% (OR 18.0, 95% CI 2.25-144.0). Patients with Hgb A1c ≥ 8.5% had higher rates of low five minute APGAR scores than patients with Hgb A1c <8.5% (11.1% vs. 2.1%, P<0.002). There was no significant difference between the two groups in adverse pregnancy outcome (OR 1.58, 95% CI 0.98-2.56) and rates of primary cesarean delivery (OR 1.16, 95% 0.64-2.11).

Conclusion: In patients with preexisting diabetes mellitus, Hg A1c ≥8.5% during early pregnancy is associated with increased risk of shoulder dystocia, lower 5 minute Apgar score and delivery at an earlier gestational age.

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High Normal Maternal Gestational Glucose Concentration and Placental Pathology. D Tate,[1] G Mari,[1] J Zhang,[2] J Samson,[1] N Schlabritz-Loutsevitch.[1] *[1]Obstetrics and Gynecology, University of Tennessee Health Science Center; [2]Pathology, University of Tennessee Health Science Center.*

Introduction: The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study found strong associations between higher normal levels of maternal glucose at 24-32 weeks of gestation and adverse pregnancy outcomes (N Engl J Med 2008; 358: 1991– 2002). Elevated maternal glucose concentrations in combination with maternal obesity have an adverse effect on the utero-placental blood flow and fetal well-being. Identification of the patients, who are at risk of the development of fetal complications, is a goal of modern obstetrical care. **The goal** of this study was to evaluate the effect of maternal gestational glucose concentrations on placental pathology in obese and non-obese patients.

Material and Methods: The retrospective analyses of placental pathology databank (2008-2010) and patients' medical histories were performed. Two hundred thirty patients met the inclusion criteria (term pregnancy, absence of existing co-morbidities, and a normal maternal gestational glucose concentration as determined after a 50-g, 1-h oral glucose challenge test at 24–32 weeks of gestation). Placental pathology, fetal and placental weight, glucose concentration after 1 hour glucola test and maternal Body Mass Index (BMI) were analyzed using ANOVA test. The differences were considered significant at $p<0.05$. **Results**: In non-obese patients (BMI ≤2 9.9 kg/m^2) a high normal maternal gestational glucose concentration (>130 mg/dl) was associated with an increased incidence of intervillous fibrinosis ($p<0.05$). **Conclusion:** This study presents a novel finding that the higher normal maternal gestational glucose levels in non-obese patients may be associated with the placental hypoxia.

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Rates of Recurrent Spontaneous Preterm Birth by Maternal Body Habitus in Women Receiving 17-α-Hydroxyprogesterone Caproate. Julia Timofeev,[1] Maisa Feghali,[1] Annelee Boyle,[1] Donna Brown,[1] Niki Istwan,[2] Debbie Rhea,[2] Rita W Driggers.[1] *[1]Obstetrics & Gynecology, Washington Hospital Center, MedStar Health, Washington, DC, USA; [2]Department of Clinical Research, Women's & Children's Health, Alere Health, Atlanta, GA.*

Objective:

To examine the influence of maternal pre-pregnancy body mass index (BMI) on rates of recurrent spontaneous preterm birth (SPTB) in women receiving 17-α-hydroxyprogesterone caproate (17P).

Methods:

Retrospective analysis of a cohort of 6,253 women with singleton gestation and prior SPTB enrolled in a 17P home administration program between 16-26 weeks was performed. Data were grouped by pre-pregnancy BMI (lean <18.5 kg/m^2, normal 18.5-24.9 kg/m^2, overweight 25-29.9 kg/m^2, and obese ≥30 kg/m^2). Maternal characteristics and delivery outcomes were compared using Pearson's χ^2 and Kruskal-Wallis test with *p*-values adjusted for multiple comparisons.

Results:

Overall, SPTB <28 weeks was significantly lower in normal weight women. The rates of recurrent SPTB were highest in the group with BMI <18.5 kg/m^2. No statistically significant difference in the rate of SPTB at <37, <34 or <28 weeks was observed for those with initiation of 17P at 16-20 weeks vs. 21-26 weeks within each BMI group.

Conclusion:

Recurrent spontaneous preterm delivery <37 weeks is more common in women with lean BMI (<18.5 kg/m^2), and lower in those with BMI ≥30 kg/m^2. All patients should be counseled on optimizing pre-pregnancy weight and appropriate weight gain during pregnancy.

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Recurrent spontaneous preterm birth by BMI in women on 17-α-hydroxyprogesterone caproate

	Lean	Normal	Overweight	Obese	*p*-value
	<18.5 kg/m²	18.5-24.9 kg/m²	25-29.9 kg/m²	≥30 kg/m²	
Overall					
SPTB<37 wks	35.8%	29.3%	27.0%[1]	25.2%[1,2]	<0.001
SPTB<34 wks	7.5%	7.7%	8.2%	8.1%	0.907
SPTB<28 wks	2.9%	1.5%	2.6%	2.9% [2]	0.006
1 prior SPTB					
SPTB<37 wks	33.2%	26.6%	24.5%[1]	22.2%[1,2]	0.001
SPTB<34 wks	6.4%	6.7%	7.6%	7.3%	0.771
SPTB<28 wks	1.8%	1.3%	2.6%[2]	2.9%[2]	0.004
2+ prior SPTB					
SPTB<37 wks	42.5%	37.2%	34.9%	33.1%	0.227
SPTB<34 wks	10.3%	10.7%	10.5%	10.1%	0.994
SPTB<28 wks	5.7%	2.1%	2.3%	2.7%	0.220
Prior SPTB<28 wks					
SPTB<37 wks	48.4%	29.8%[1]	25.7%[1]	24.2%[1]	<0.001

SPTB – spontaneous preterm birth. Adjusted $p<0.05$ vs. [1]lean, [2]normal.

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Early Platelet Dysfunction in Prepregnancy Diabetes before the Development of Preeclampsia. Rita Zafra,[1] Julia Malis,[1] Laura Londra,[1] Maria Small,[2] Mike Kruger,[1] Ray Bahado-Singh.[1] [1]*School of Medicine, Wayne State University, Detroit, MI, USA;* [2]*School of Medicine, Duke University, Durham, NC, USA.*

Background: Pre-pregnancy diabetes mellitus (PPDM) is associated with an increased risk for the development of PE. Significant platelet activation is known to occur in diabetes as well as in preeclampsia (PE). Our objective was to determine whether there was early evidence of platelet dysfunction in PPDM patients destined to develop PE later in pregnancy.

Study Design: Platelet count (PC) and mean platelet volume (MPV) were measured at < 20 weeks, prior to development of PE. We studied 3 groups: PPDM with subsequent development PE (PPDM/PE), PPDM without later PE and normal controls without PPDM or later PE. ANOVA was used to compare PC and MPV in the three groups. PC, MPV and dipstick protein levels measured before 20 weeks were included in a logistic regression to determine whether platelet indices robustly correlated with the subsequent development of PE. Odds ratio (OR) and p-values were calculated with $p < 0.05$ considered significant.

Results: There were 126 PPDM/PE, 150 PPDM without later PE and 150 normal control cases. Mean maternal ages were 29.6 (6.3), 29.3 (5.8) and 24.6 (4.7) years ($p< 0.001$) respectively. PC (mean/SD) (in thousands) were significantly different for the groups PPDM/PE-300.5(84.7), PPDM -291.6 (69.9), and 264.3 (67.7) for controls, $p < 0.001$. MPV was not significantly different between groups ($p= 0.85$). Logistic regression analysis revealed early PC measurements significantly correlated with the later development of PE ($p< 0.03$) as did dipstick proteinuria: trace ($p = 0.01$) and $\geq 1^+$ ($p < 0.001$) but not MPV ($p = 0.91$).

Conclusion: Based on a large number of cases, we have found evidence of platelet activation well before PE development in PPDM cases. In contrast to the increase in MPV previously reported for non-diabetic patients destined to develop PE, we found no change in MPV. Rather, a significant increase in PC was noted to precede PE development in PPDM. The increased platelet count could represent an early compensatory response to evolving platelet dysfunction.

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Meta-Analysis of Transcriptome Data Reveals Novel Molecular Pathways Related to Preeclampsia. Gijs Afink,[1] Miranda van Uitert,[1] Perry Moerland,[2] Joris van der Post,[3] Carrie Ris-Stalpers.[1,3] [1]*Reproductive Biology Laboratory, Academic Medical Center, Amsterdam, Netherlands;* [2]*Clinical Epidemiology, Biostatistics, and Bioinformatics, Academic Medical Center, Amsterdam, Netherlands;* [3]*Obstetrics and Gynecology, Academic Medical Center, Amsterdam, Netherlands.*

Introduction: Transcriptome analysis is an important tool to identify differential gene expression between normotensive and preeclamptic (PE) placentas. Some key targets involved in PE pathogenesis (FLT1, ENG) were discovered using this approach. Because of relatively small sample size, variability in platforms, patient characteristics and statistical methods used, no clear PE-specific gene expression consensus profile has been defined yet.

Methods: From PubMed and GEO 24 datasets were identified describing whole genome mRNA expression data interrogating PE. 8 of them were retrieved from GEO, 7 were kindly provided by authors, 3 are in the process of being provided by the authors, 2 authors refused to collaborate, and 4 did not respond. Datasets were compared using the method described by Ramasamy et al., PLoS Med 5:e184, 2008.

Results: Initial meta-analysis of several publically available datasets shows that combining data results in an increased number of differentially expressed genes as compared to individual datasets. In addition, some individual datasets have an aberrant gene expression pattern compared to that generated by meta-analysis. These are not only data sets with an obviously different sampling location (CVS) compared to the most common location (villous biopsy), but also seemingly homogeneous datasets.

Discussion: Our meta-analysis shows that combining multiple small-size transcriptome studies results in an increased statistical power generating a more robust PE differential gene expression pattern. In addition, it provides a tool to assess overlap and differences in gene expression between samples from different locations. The approach both confirms already known molecular pathways related to PE, but also generates novel leads for both functional and biomarker studies related to PE.

As a next step we aim to build a network of research groups that will share transcriptome and detailed patient data enabling robust linkage of gene expression patterns to clinical outcome that in a consortium form can all benefit from increased power of their study.

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Sequencing Analysis of NLRP7 and NLRP2: Can Their Mutations Cause Infertility or Are They Specific Only for Gestational Trophoblastic Disease? Lusine Aghajanova,[1] Sangeetha K Mahadevan,[1] Signe Altmae,[2] Anneli Stavreus-Evers,[3] Ignatia B Van den Veyver.[1] [1]*Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA;* [2]*Department of Clinical Science, Intervention and Technology, Division of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden;* [3]*Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden.*

Introduction: Hydatidiform mole (HM) is an abnormal human pregnancy with cystic degeneration of chorionic villi. Most complete HM cases are androgenetic and sporadic (AnCHM), but rare recurrent and sometimes familial cases are biparentally inherited (BiHM). Mutations of *NLRP7* were identified in a subset of affected women with recurrent BiHM. It has recently been reported that heterozygous mutations in *NLRP7* might cause other forms of reproductive failure, such as recurrent miscarriage. Considering that the defect underlying BiHM may be defective reprogramming of imprinting in germ-cells or its maintenance in the early embryo, we hypothesized that mutations in *NLRP7* or its ancestral gene, *NLRP2*, which has also been associated with an imprinting disorder in offspring, may be associated with unexplained infertility. To address this hypothesis, we performed mutation analysis of *NLRP7* and its ancestral gene, *NLRP2*, in a well-defined group of women with unexplained infertility.

Materials and Methods: Genomic DNA was isolated from peripheral blood samples of 94 women with unexplained primary infertility (age 33.0 ± 3.5years). All women were of Swedish-Finnish origin. Eleven coding exons and exon-intron boundaries of the *NLRP7* gene were sequenced in all affected women. Sanger sequencing was carried out at Beckman Coulter Genomics. The chromatograms were analyzed with Sequencher® version 4.7 sequence analysis software. Identified sequence variants were compared with those already in the 1000 Genomes, dbSNP and HapMap databases. Similar analysis of *NLRP2* gene is currently ongoing.

Results: Sequence analysis of *NLRP7* demonstrated no putative disease-causing mutations in 11 coding exons and exon-intron boundaries. Several single-nucelotide polymorphisms (SNPs) were identified and compared with the frequency of reported SNPs in general population.

Conclusions: These results support the hypothesis that mutations in *NLRP7* may be specific for BiHM. However, larger studies in women with other forms of reproductive failure, such as recurrent pregnancy loss, need to be done.

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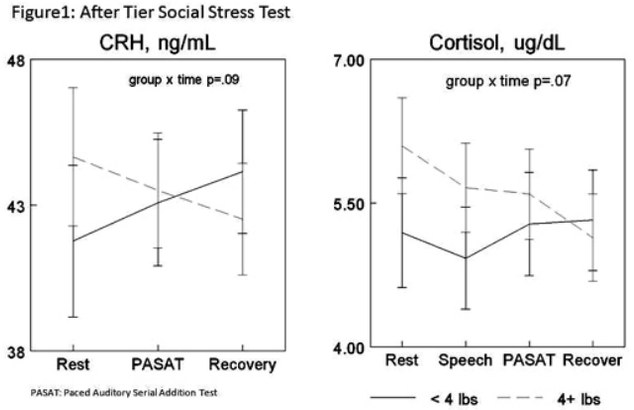
Postpartum Weight Retention and HPA-Axis Response to Stress. Carmen J Beamon,[1] Samantha Meltzer-Brody,[2] Karen Grewen,[2] Alison M Stuebe.[1] [1]*Obstetrics & Gynecology, University of North Carolina, Chapel Hill, NC, USA;* [2]*Psychiatry, University of North Carolina, Chapel Hill, NC, USA.*

Background: Postpartum depression is associated with increased postpartum weight retention. Several authors have suggested that differences in HPA-axis regulation during the postpartum period may mediate this association. We hypothesized that postpartum weight loss would be associated with differences in HPA-axis response to a standardized social stressor.

Methods: We performed a secondary analysis of data from a longitudinal cohort study of perinatal depression and lactation. We measured BMI and depression and anxiety symptoms at 2 and 8 weeks postpartum. At the 8 week visit, we administered the Trier Social Stress Test (TSST) and obtained blood levels for cortisol and CRH. We used Fisher's Exact tests and Pearson Correlations to measure associations between mood history and weight change. We used mixed repeated measures analysis to compare neuroendocrine markers among women with weight loss above or below the median from 2 to 8 weeks postpartum. Main effect p values <.05 and interaction p values <.1 were considered statistically significant.
Results: Weight change data were available for 37 breastfeeding participants at the 8 week visit. We found no association between weight loss and mood symptoms. In mixed effect models, we found a significant interaction between weight change group (≥ 4lbs vs.<4lbs) and time for both CRH and cortisol, adjusting for BMI at visit 2, parity, current antidepressant use, breastfeeding intensity, and depression and anxiety symptoms (Figure 1). Among women who lost ≥4 lbs, cortisol and CRH levels declined during the TSST. Among women who lost <4lbs, CRH levels increased and cortisol levels did not change.
Discussion: Contrary to earlier reports, we did not find an association between weight change and mood symptoms. However, weight loss during the first 8 weeks after birth was associated with differences in trajectory of CRH and cortisol during the TSST. These results support our hypothesis that differences in HPA-axis regulation may be associated with postpartum weight retention.

Figure1: After Tier Social Stress Test



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Sex Steroids Offer Minor Suppression of Fatty Acid Uptake in Male Placentas. Elizabeth Brass,[1] Kent L Thornburg,[2,3] Perrie F O'Tierney.[3] *[1]Dept OB/GYN, Oregon Health & Science University, Portland, OR, USA; [2]Dept Med (Cardiovasc Med), Oregon Health & Science University, Portland, OR, USA; [3]Heart Research Center, Oregon Health & Science University, Portland, OR, USA.*
OBJECTIVE: The fetus is dependent on the placenta for its supply of long chain polyunsaturated fatty acids (LCPUFA), which are essential in fetal growth and development. We have found sex specific differences related to placental fatty acid uptake: male placentas of normal BMI women had greater uptake and it is dramatically suppressed in maternal obesity, while female uptake is unaffected by maternal BMI. Given that steroid hormone levels are similar between males and females at birth; we hypothesized that the sex steroid environment plays a minor role in regulating fatty acid transport.
METHODS: Women were recruited upon admission for cesarean section (n=25). At delivery, placental explants were treated with physiologic concentrations of estradiol (175ng/dL) and testosterone at either low (33ng/dL) or high (500ng/dL) concentrations. Fatty acid uptake using ^{14}C-labelled oleic acid (OA), arachidonic acid, (AA) and docosahexanoic acid (DHA) was measured after 1, 4, and 24 hours of treatment and uptake calculated as nmol fatty acid/mg protein at 15 minutes. Results were stratified by fetal sex and maternal first trimester BMI (normal BMI<25 or obese BMI<26) and women with co-morbidities were excluded. Dichotomous outcomes were analyzed using 1 way ANOVA followed by Tukey post-hoc testing; $p<0.05$ was used to indicate statistical significance.
RESULTS: Placental fatty acid uptake in females was unchanged with any treatment and among BMI groups. In males from normal BMI women, placental fatty acid uptake of OA and DHA was decreased after treatment with testosterone at low and high concentrations at two time points, either 1 or 24 hours ($p<0.05$). In males of obese women, OA uptake was reduced after treatment with estradiol and testosterone at 1 hour ($p<0.05$). Sex hormone levels were not different between males and females at birth (estradiol: male 46-50, female 50-54ng/dL; testosterone: male 67-390, female 42-587ng/dL).
CONCLUSION: Steroid sex hormones had no effect on fatty acid uptake of LCPUFA in female placentas, but had a measurable effect on two fatty acids in male placentas. While fatty acid uptake was affected by exposure to sex steroids in male placentas, this response does not appear to explain the dramatic sex specific differences in placental LCPUFA uptake.

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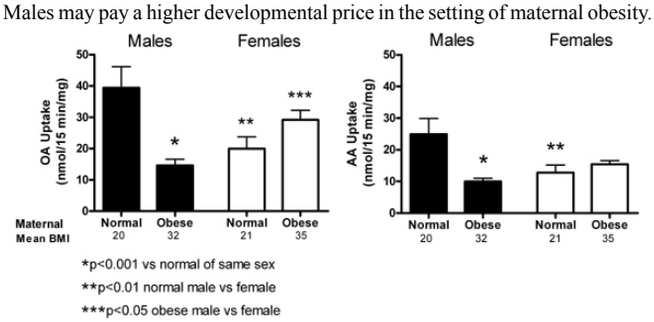
Maternal Obesity Suppresses Male Dominance of Placental Fatty Acid Uptake. Elizabeth Brass,[1] Kent L Thornburg,[2,3] Perrie F O'Tierney.[3] *[1]Dept OB/GYN, Oregon Health & Science University, Portland, OR, USA; [2]Dept Med (Cardiovasc Med), Oregon Health & Science University, Portland, OR, USA; [3]Heart Research Ctr, Oregon Health & Science University, Portland, OR, USA.*
OBJECTIVE: The fetus is dependent upon the placenta for its supply of long chain polyunsaturated fatty acids (LCPUFA), which are essential in fetal growth and development. Previous work suggests that males have greater fatty acid requirements and placental uptake than females during development. We hypothesized that male placental fatty acid uptake would be more sensitive to maternal body mass index (BMI) compared to females.
METHODS: Women were recruited upon admission to Labor & Delivery for cesarean section (n=25). At delivery, placental samples were collected for fatty acid uptake studies using ^{14}C-labelled oleic acid (OA), arachidonic acid, (AA) and docosahexanoic acid (DHA) in placental explants. Uptake was calculated as nmol fatty acid/mg protein at 15 minutes. Results were stratified by fetal sex and maternal first trimester BMI (normal BMI<25 or obese BMI>26). Women with significant co-morbidities were excluded. Dichotomous outcomes were analyzed using 1 way ANOVA followed by Tukey post-hoc testing; $p<0.05$ was used to indicate statistical significance.
RESULTS: Placental fatty acid uptake of OA and AA in males of obese women was decreased 62% and 60% respectively compared to normal BMI women ($p<0.001$). In females, placental fatty acid uptake was not suppressed in the setting of obesity (Figure1). Placental fatty acid uptake of OA and AA in females of normal BMI women was reduced 49% and 48% respectively compared to its male cohort ($p<0.01$). There was no difference in DHA uptake between sex or BMI groups.
CONCLUSION: Male placentas of normal weight women took up LCPUFAs at a higher rate than female placentas per gram of tissue. As predicted, placentas from males with obese mothers had suppressed uptake of two LCPUFAs, while uptake in females was unaffected by maternal BMI. This data suggest that males born to high BMI mothers may have inadequate LCPUFA acquisition. Males may pay a higher developmental price in the setting of maternal obesity.



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Maternal IL-8 Concentration and the Key Molecule of the Placental Endocannabinoid System. Brian E Brocato,[1] Cezary Skobowiat,[2] Andrzej Slominski,[2] Giancarlo Mari,[1] Peter W Nathanielsz,[5] Kulkarni Anand,[2] Gene B Hubbard,[3] Edward J Dick, Jr,[4] Natalia Schlabritz-Loutsevitch.[1] *[1]OBGYN, Univ of Tennessee Health Sciences Center, Memphis, TN, USA; [2]Pathology, Univ of Tennessee Health Sciences Center, Memphis, TN, USA; [3]Pathology, Univ of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [4]Pathology, Texas Biomedical Research Institue, San Antonio, TX, USA; [5]OBGYN, Univ of Texas Health Science Center at San Antonio, San Antonio, TX, USA.*
Introduction: Maternal obesity (MO) is a major risk factor for adverse fetal outcomes. The mechanism behind this is unknown. MO is associated with systemic and placental inflammatory response although inflammatory cytokines (CK) do not cross the syncytiotrophoblast (ST) barrier. The endocannabionoid system (ECB) is part of the CK network regulating early placentation. In term pregnancy, the role of ECB in association with CK function has not yet been studied.

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We hypothesize that imbalance of CK in MO will result in an increase placental ECB tone, linking maternal inflammation and placental metabolic status.
The aim of this study was to evaluate the protein expression of fatty acid amide hydrolase (FAAH), an ECB cleavage enzyme and its biological function in term placenta in relation to CK in MO.
Material and Methods: Placenta, maternal and fetal serum from four obese and four non-obese baboons (Papio spp.) were collected at term and processed; leptin, VEGF, IL-8, IL-6, IL-1beta, TNF alfa and adiponectin concentrations were estimated as previously described (Placenta;30(9):752-60). FAAH expression was demonstrated by immunohistochemistry. Slides were digitized using ScanScope® XT at 0.25 µm/pixel. The images were viewed and analyzed using ImageScope™ v11.1.2.752. Two tailed student t-test and correlation were applied for statistical analysis.
Results: FAAH expression was identified in syncytiotrophoblast (ST), extra-villious trophoblast and macrophages. There were no differences in ST FAAH expression between the two groups. The maternal concentration of IL-8 correlated negatively with ST FAAH expression ($r = -0.76$, $p = 0.03$).
Conclusion: Placental ST ECB may translate selected maternal CK signaling into the placental metabolic response, therefore providing a potential target for intervention to improve placental function and fetal outcomes in conditions associated with maternal inflammatory status. HD 21350, P51 RR013986, 1R01AR056666-01A2

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Spontaneous Abortion Is Associated with Lower Levels of Decidual Intermedin (IMD) during 1st Trimester in Human Pregnancy. Madhu Chauhan, Dara Havemann, Meena Balakrishnan, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
The invasion of trophoblasts into the endometrium is crucial to placentation and successful pregnancy. Defective placentation is thought to be the main source of several complications in pregnancy which originate mainly due to an imbalance in the expression of pro-invasive and anti-invasive factors expressed in deciduas and villi. Intermedin (IMD)/adrenomedullin2 is a novel calcitonin (CT)/calcitonin gene-related peptide family peptide discovered in 2004. We showed earlier that IMD downregulates expression of invasion inhibitory factor CD82 in 1st trimester human decidual tissue. Therefore, this study was undertaken to assess the expression profile of IMD in decidual tissues obtained from elective abortions at different weeks during 1st trimester of human pregnancy and assess if there are any pathology related changes in decidual expression of IMD in 1st trimester spontaneous abortions.
Methods: These studies were approved by the Institutional Review Board at the UTMB. Informed consent was obtained from all patients. Decidual tissue was carefully dissected free of villous material from abortion tissues obtained at 9-14 weeks of gestational age from women undergoing spontaneous abortions and from women who opted for abortion. Tissues were flash frozen at -80ºC until used for total RNA extraction using QIAGEN RNA extraction kit and qRT-PCR analysis. Expression of mRNA was analyzed relative to that of GAPDH expression.
Results: 1) Expression of IMD mRNA is 100 fold lower in decidual tissue obtained from spontaneous compared to elective abortions in 1st trimester human pregnancy ($P<0.05$, ($N=12$-20), 2) Levels of IMD mRNA are higher in 6 weeks deciduas and shows a trend of decline as the pregnancy progresses towards 2nd trimester and, 3) Immunofluorescent studies using IMD antibody shows an increased expression of IMD immunoreactivity in 7 weeks compared to the 10 weeks decidual tissue sections obtained from electively aborted human placenta.
Conclusion: Association of spontaneous abortion with 100 fold down regulation of decidual IMD in 1st trimester human pregnancy suggests potential involvement of this novel peptide in the pathophysiology of human pregnancy.

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Expression of Mucin1 (MUC1) in Human Decidual Stromal Cells and Its Regulation by Intermedin (IMD). Madhu Chauhan, Meena Balakrishnan, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
MUC1 regulates adhesion of trophoblasts to endothelial cells and their trans-endothelial cell migration. We have shown that CT/CGRP family peptide Intermedin (IMD) is expressed in human placenta and increases trophoblast invasion and migration in 1st trimester pregnancy. Invading trophoblast cells are under constant influence of decidual stromal cells (DSCs). MUC1 is expressed in deciduas and is shown to co-localize with invaded cytotrophoblast cells in decidua. However, it is not known if DSCs express MUC1 and if decidual MUC1 is regulated by IMD and sex steroid hormones.
Objective: To assess, 1)expression of MUC1 in DSCs isolated from 1st trimester electively aborted placenta, 2)effect of IMD on expression of MUC1 in decidual explants, 3)effect of IMD on regulation of MUC1 in DSCs and 4) effect of 17β-estradiol and progesterone on the expression of decidual MUC1.
Methods: This study was approved by the Institutional Review Board at the University of Texas Medical Branch at Galveston. Decidual tissue was isolated from 1st trimester electively terminated pregnancies (6-11 weeks) and used for explants culture and DSC isolation. DSCs were cultured in DMEM containing 10% normal or charcoal stripped FBS. Total RNA was isolated by Qiagen RNA isolation kit. Decidual explants and DSCs were serum starved overnight before treating with IMD in presence or absence of IMD antagonist or with 17β- estradiol and progesterone alone (10^{-8}M) or in combination for 24 hrs. QRT-PCR analysis and DNA sequencing was done to assess the expression and identity of PCR amplified MUC1 in DSCs respectively.
Results: 1) MUC1 mRNA as well as protein is expressed in DSCs as demonstrated by DNA sequencing and western blot analysis, 2)IMD significantly decreases the expression of MUC1 in 1st trimester decidual explant cultures ($P<0.05$),3) IMD decreases expression of MUC1 and IMD antagonist $CGRP_{8-37}$ inhibits IMD effects in DSCs from 1st trimester electively aborted placenta($P<0.05$) and 4) Treatment with 17β-estradiol and progesterone alone have no effect but 17β-estradiol and progesterone together significantly increases the expression of MUC1 mRNA in decidual stromal cells ($p<0.05$).
Conclusion: This study demonstrates that MUC1 is expressed in decidual stromal cells in first trimester human placenta and that expresion of decidual MUC1 is suppressed by IMD.

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Knockdown of RAMPs in Rat Vascular Smooth Muscle Inhibits Cell Surface Expression of Adrenomedullin Receptor Complex ($RAMP_3$/CRLR) and Cell Migration. Madhu Chauhan, Uma Yallampalli, Meena Balakrishnan, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
Studies performed by over expressing receptor activity modifying proteins $(RAMP)_2$ and $RAMP_3$ in vascular smooth muscle cells (SMC) of aorta have shown that anti- migratory effect of CT/CGRP family peptide Adrenomedullin (AM) are mediated through $RAMP_2$ and $RAMP_3$.This study was undertaken to assess the effect of blocking the synthesis of Ramp1, Ramp2 and Ramp3 on migration and cell surface expression of AM receptor in mesenteric artery SMCs.
Objective:1) Demonstrate the functional AM receptor complex in rat mesenteric artery SMCs Using proximity ligation assay,2) create stable knockdowns of $RAMP_1$, $RAMP_2$ and $RAMP_3$ in rat mesenteric artery SMCs and assess the effect of $RAMP_1$, $RAMP_2$ and $RAMP_3$ knockdown on cell surface expression of AM receptor and 3) Assess the effect of $RAMP_1$, $RAMP_2$ and $RAMP_3$ knockdown on mesenteric artery SMC migration.
Methods: Vascular smooth muscle cells were isolated from mesenteric artery as per the published method. Cells at passage 9 were used for creating knockdown of $RAMP_1$, $RAMP_2$ and $RAMP_3$ with gene specific shRNA (Origene) and sh-scramble served as control. Puromycin was used for stable selection of transfected cells. Scratch assay was used for migration studies and analyzed by NIH image J software. Cell surface expression of ADM receptor was assessed by Proximity ligation assay performed with untransfected and shRNA transfected cells.
Results:1)There exists a basal cell surface expression of AM receptor in resting primary rat mesenteric artery SMCs,2)Acute stimulation of mesenteric artery SMCs with AM(10^{-8}M) for 2 minutes causes an increase in cell surface expression of $RAMP_3$/CRLR receptor complex compared to $RAMP_2$/CRLR or $RAMP_1$/CRLR receptor complex, 2)knockdown down of $RAMP_3$ causes a significant reduction in the cell surface expression of AM receptors compared to the cells with $RAMP_1$ or $RAMP_2$ knockdown and, 3) Knockdown of $RAMP_1$, $RAMP_2$ or $RAMP_3$ inhibits migration of mesenteric artery SMCs in the order where $RAMP_1$>$RAMP_2$=$RAMP_3$ **Conclusion:** 1)AM induced acute stimulation of cell surface expression of $RAMP_3$/CRLR receptor complex and its inhibition by $RAMP_3$ knockdown suggests $RAMP_3$/CRLR to be the primary functional AM receptor in rat mesenteric artery; and 2) Migration of rat mesenteric artery SMCs is inhibited by blocking the synthesis of $RAMP_1$, $RAMP_2$ or $RAMP_3$.

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Assessment of Pre-Analytical Factors and Quality Measures for RNA Isolation from Human Placenta. Amy L Creekmore, Kerry L Sanders, Robinah K Maasa, Men-Jean Lee, Jill L Reiter. *Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA.*

Objective: High quality RNA is of paramount importance in accurately interpreting gene expression changes in the placenta throughout pregnancy, as well as in common placental pathologies such as preeclampsia, and intrauterine growth restriction (IUGR). The purpose of this study was to develop standard operating procedures for the collection of human placental tissue and for the isolation of high quality RNA for performing reproducible pregnancy-related molecular studies.

Study Design: We optimized and compared several different parameters to minimize both mechanical and enzymatic RNA degradation. These parameters included vaginal vs. cesarean delivery, length of tissue storage at 4°C, tissue preservation in liquid nitrogen vs. RNA*later*, tissue disruption by mortar and pestle vs. homogenizer, and RNeasy vs. Trizol RNA isolation kits. RNA quality (RQI), purity (260/280 absorbance), yield, and integrity (5'/3' mRNA end degradation) were assessed. RNA purity was also assessed for the absence of PCR inhibitors by spiking real-time quantitative PCR assays with a synthetic template (SPUD assay). 5'-3' assays were performed by comparing differences in Cq values for primer sets located near 5' and 3' mRNA ends.

Results: We performed 150 RNA isolations from 30 term placentas. The overall yield was 365 ± 197 ng RNA per mg of tissue. The A260/A280 ratio for all samples was 2.11 ± 0.1 (mean ± s.d.) and the RQI was 7.1 ± 1.4. No significant differences in RNA purity, yield, or quality were observed amongst different placental collection or RNA isolation techniques. However, poor RQI values of 2.7 to 3.3, indicating degraded RNA were obtained after brief thawing of frozen placental samples. Expression levels of common reference genes did not vary significantly amongst the samples (ACTB, Cq = 21 ± 0.7 and TBP, Cq = 25.9 ± 0.7). Additional experiments are underway to assess changes in more labile mRNA species.

Conclusion: High quality RNA can be isolated from human placentas using a wide variety of techniques and is not dependent on the mode of delivery, or length of storage at 4°C (1-24 hrs). We found that the single most important factor in isolating intact RNA was the speed in which frozen tissue was disrupted. The results of these studies will be useful for establishing standard procedures for placenta collection for pregnancy biobanks.

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Comparison of Negative Immunoselection Methods To Purify Villous Cytotrophoblast Cells. Carla Fortique,[1] Ranjan Upadhyay,[1] Audrey Hertenstein,[1,2] Rona S Carroll,[1] Ursula B Kaiser,[1] Wendy Kuohung.[1,2] *[1]Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA, USA; [2]Obstetrics and Gynecology, Boston University School of Medicine, Boston, MA, USA.*

Objective: When isolating human placental cytotrophoblast cells (CTBs), the presence of contaminating cells such as fibroblasts and leukocytes is a common problem. Our aim was to identify a cost-effective and efficient immunomagnetic purification protocol that would allow us to obtain highly pure CTBs from placentas of various gestational ages.

Methods: Placentas were collected under an IRB-approved protocol. We performed enzymatic digestion of dissected villous placental tissue, followed by Percoll gradient separation. For the purpose of isolating CTBs using immunomagnetic separation, we compared the Dynabeads® (Invitrogen) and EasySep® (StemCell) systems. Both methods are based on positive labeling using biotinylated antibodies to CD45 and CD9 for leukocytes and fibroblasts, respectively. The CTBs are collected by negative selection: after placement in the magnet, unlabeled CTBs are poured off while labeled leukocytes and fibroblasts are immobilized by magnetic particles. Suggested primary antibody amounts for 100 million cells are 10 µg for Dynabeads and 1.5µg for EasySep. The EasySep reagent targets biotin for selection and allows the magnetic particles to bind to labeled cells. The Dynabeads system allows antibodies to be preconjugated to magnetic beads. Purity was assessed using staining against cytokeratin-7 (CK7), a protein specific for trophoblast cells.

Results: In divided CTB fractions from the same placenta, we found that both the Dynabeads and EasySep systems provided the same final cell yield. Both Dynabeads (95.4 ± 1.2%) and EasySep (96.0 ± 2.3%) resulted in similar high purity as measured by positive CK7 staining compared to CTBs that had not undergone immunomagnetic separation (86.8 ± 3.2%). The Dynabeads protocol on average took about 25 additional minutes to complete compared to the EasySep protocol.

Conclusions: Both negative immunoselection methods utilizing the Dynabeads and EasySep systems yield highly pure villous CTB fractions. Both methods result in similar loss of cells, but the Dynabeads protocol requires 6.7 times more antibody than the EasySep protocol. Therefore, we conclude that EasySep is a more affordable and less time consuming immunomagnetic separation method for purification of CTBs.

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Effect of Obesity on Mitochondrial Function and Hypoxia Susceptibility in Human Syncytiotrophoblasts. J Mele, A Maloyan, L Myatt. *CPNR, Department of OB/GYN, UTHSC, SA, TX, USA.*

Obesity in pregnancy is associated with increased morbidity in the perinatal period and with programming of the offspring for disease in adult life. Obesity has been recently linked to mitochondrial dysfunction across a variety of tissues (e.g., skeletal muscle and liver), and oxidative stress has been proposed to explain this dysfunction. The placenta has high metabolic activity and may be susceptible to the chronic inflammatory and oxidative milieu of obesity. **We hypothesized that placental mitochondrial respiratory function will be reduced in women with increasing BMI.** Primary cytotrophoblasts were isolated from placentas collected by c-section (no labor) at term from women (n=10) with a range of BMI (25-45) from lean (LN) to obese (OB). Cytotrophoblasts were isolated and syncytialized over 60 hrs. Trophoblast mitochondrial respiration was measured using a XF24 analyzer (Seahorse Bioscence). Basal oxygen consumption rate (OCR) was measured and the respiratory parameters - maximum respiration, spare capacity and coupling efficiency - were calculated using specific mitochondrial inhibitors oligomycin, FCCP and rotenone/antimycin A. When plotted against maternal BMI, all parameters, except coupling efficiency, showed a significant inverse correlation with increasing maternal BMI (Basal Respiration, R^2=0.6; Maximum respiration, R^2=0.48; Spare capacity, R^2=0.37 $p<0.05$). Thus, placental mitochondrial function is compromised with increased maternal BMI supporting the concept that the underlying pathology from maternal obesity and its associated negative effects on the fetus results from deficits in energetic capacity. We investigated the sensitivity of syncytiotrophoblasts to hypoxia, a known stressor involved in obesity. Syncytialized cells were treated for 24hrs with increasing concentrations of desferrioxamine (DFO; 25-200µM), an iron chelator that mimics hypoxia via HIF1α stabilization. Regardless of BMI, DFO significantly reduced maximum respiration and spare capacity in a concentration-dependent manner. The IC50 for maximum respiration was 2-3 fold lower for the syncytiotrophoblasts derived from OB relative to LN women. Syncytiotrophoblasts from obese mothers are functionally impaired under basal conditions and more sensitive to adverse hypoxic conditions. This data agrees with our other studies showing reduced expression of mitochondrial complex proteins and transporters in placentae from OB mothers.

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Effect of Obesity and Fetal Gender on Expression of Mitochondrial Electron Complexes in Human Placental Villous Tissue. J Mele, A Maloyan, L Myatt. *CPNR, Department of OB/GYN, UTHSCSA, SA, TX, USA.*

Obesity during pregnancy is linked to programming of the offspring for development of obesity and metabolic syndrome in later life. Obesity is linked to chronic inflammation and mitochondrial dysfunction via production of reactive oxygen species. Gender related differences in placental physiology are well known and the male fetus are sensitive to intrauterine stress and has poorer perinatal outcomes. Little is known about alterations in mitochondrial energetics occurring in the placenta with increasing body mass index (BMI) and the confounding fetal (and placental) gender. We hypothesised that there are both obesity related and gender specific differences in gene expression of mitochondrial electron transport chain subunits in the placenta. Placentae were collected by C-section (no labor) at term from lean, overweight and obese women with either a male or female fetus (n=4/group). cDNA was prepared from villous tissue and expression of 84 genes involved in oxidative phosphorylation was determined using the Mitochondrial Energy PCR Array System (SaBiosciences). Differences in gene expression in overweight and obese groups relative to the lean group were calculated for male and female tissues. Comparisons between genders in the lean tissues revealed that placentae from a female fetus displayed a 1.5x higher expression of complex V assembly factor OXA1L, and 1255x lower expression of Cox6C (complex IV). With increasing BMI, placentae from males were found to downregulate ATP synthase F1/Fo subunits (complex V), Cox subunits (complex VI), and NADH dehydrogenase subunits (complex I) by 1.2-1.7 fold ($p<0.05$). More genes were affected in overweight vs lean compared to obese vs lean in males,

suggesting adaptation/dysfunction in the placenta. In contrast, with increasing BMI, placentae from a female fetus showed increased expression of complex V (ATP6V1E2) and complex IV (COX6A2 and 6C) subunits. Decreased expression of complexes V and IV proteins measured by Western Blot correlated with the male array data. Placental ATP levels were 2.5x lower in males in obese group vs. lean whereas female ATP levels remained unaffected. Thus, not only are placentae from a female fetus energetically different from males, but there is a sexually dimorphic response to increasing BMI, with males adopting a strategy to maximize growth (down regulation of mitochondrial complex subunits) while females may adapt in utero (upregulation) to adverse conditions.

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Mechanical and Receptor-Mediated Responses of Placental Chorionic Plate Arteries Are Altered in Fetal Growth Restriction. Tracey A Mills, Susan L Greenwood, Edward D Johnstone, Colin P Sibley, Mark Wareing. *Maternal and Fetal Health Research Centre, The University of Manchester, United Kingdom.*

Background: Fetal growth restriction (FGR) is a significant cause of poor pregnancy outcomes and has negative health implications for survivors. FGR is associated with reduced placental blood flow, secondary to increased placental vascular resistance. The underlying mechanisms are incompletely understood, but abnormal structure and /or function of small fetoplacental blood vessels may contribute.[1]

Aim: To assess stretch and agonist-induced responses of small chorionic plate arteries (CPAs) arteries in normal versus FGR pregnancies.

Method: Placentas were obtained post-delivery (vaginal or Caesarean section) following a) uncomplicated pregnancies (N=5) and b) pregnancies complicated by FGR (N=6; customized birthweight centile ≤5). CPAs were dissected from biopsies stored in ice-cold HCO_3^--buffered physiological salt solution, mounted onto a wire myograph, normalised at 0.9 of $L_{5.1kPa}$ (classical normalisation; CN)[2], reset to the internal circumference at which passive tension was first measurable (L_0) and equilibrated (37°C; 20 mins; $5\%O_2/5\%CO_2$). Passive tension was recorded, the vessel exposed to a maximal constrictive dose of U46619 (10^{-6}M; TXA_2 mimetic) and data collected for total tension (active + passive tension). Post wash, arteries were stretched and the procedure repeated (6-8 stretches).

Results: Arterial diameters (by CN) were greater in FGR than uncomplicated pregnancies (Mean±SEM; 342±13μM vs.226±14μM $p<0.05$, t-test). Arterial passive tension-stretch data were fitted to exponential curves [$Y=Y0*\exp(k*X)$]; Tau (1/k) was greater in FGR [median(range); 420(290-789) vs. 330(238-662) μM, $p<0.05$ Mann Whitney U test]. Peak active tension (PAT) and area under curve (AUC) were used to assess active tension-stretch data. AUC/PAT were unaltered in FGR, however PAT, as % of CN diameter, was significantly reduced in FGR (Mean±SEM; 137±6% vs. 174±11%, $p<0.05$; t-test).

Conclusion: In FGR, small CPA passive tension-stretch characteristics are altered. Despite increased arterial diameter in FGR, PAT was unaltered and PAT diameter was significantly shifted towards the diameter determined by CN. These data suggest modified mechanical and receptor mediated responses in CPAs may contribute to altered vascular resistance and blood flow in FGR.

1.Challis et al (2000) Pediatr Res 47:309-15
2.Wareing et al (2002) Placenta 23:400-409

Support: Action Medical Research

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Circulating miRNA-323-3p Is a Novel Biomarker To Predict Ectopic Pregnancy. Zhen Zhao,[1] Qiuhong Zhao,[2] Joshua Warrick,[1] Christina Lockwood,[1] Alison Woodworth,[3] Ann M Gronowski1,[1,2] Kelle H Moley.[2] *[1]Department of Pathology and Immunology, Washington University, St. Louis, MO, USA; [2]Department of Obstetrics and Gynecology, Washington University, St. Louis, MO, USA; [3]Department of Pathology, Vanderbilt University, Nashville, TN, USA.*

BACKGROUND: Over 20 serum biomarkers have been investigated to date in an attempt to allow earlier and more accurate diagnosis of ectopic pregnancy (EP), however, none have been put into routine clinical use except for serial hCG measurements. Pregnancy-associated circulating miRNAs are proposed to serve as potential biomarkers for the diagnosis of pregnancy-associated complications. The altered expression pattern of pregnancy-associated miRNAs may reflect tissue-specific pathologic states during the ectopic pregnancy.

METHODS: A retrospective analysis was performed on a cohort of 89 women who presented to the emergency department (ED) with vaginal bleeding and/or abdominal pain/cramping and were diagnosed with viable intrauterine pregnancy (VIP), spontaneous abortion (SA), or EP. Serum hCG and progesterone concentrations were determined by immunoassays. Serum miR-323-3p, miR-517a, miR-519d, and miR-525-3p concentrations were measured using TaqMan real-time PCR. Statistical analysis was performed to determine the clinical utility of these biomarkers as single measurement test and as a multimarker panel test for EP.

RESULTS: Concentrations of hCG, progesterone, miR-517a, miR-519d, and miR-525-3p were significantly lower in EP and SA than in VIP. In contrast, the concentrations of miR-323-3p was significantly elevated in EP as compared to SA and VIP. Compared to the other single marker measurement, miR-323-3p had the highest sensitivity of 37.0% (at a fixed specificity of 90%). Comparatively, combined hCG, progesterone, and miR-323-3p panel yielded the highest sensitivities of 77.8% (at a fixed specificity of 90%). Combining hCG, progesterone, and miR-323-3p had a sensitivity of 77.8% (at a fixed specificity of 90%) and combining hCG, progesterone and all four miRNAs yielded the greatest clinical utility with 90% sensitivity and 80.7% specificity.

CONCLUSIONS: Serum miR-323-3p is a novel marker with higher serum concentrations in EP than that in both SA and VIP. As a single marker, miR-323-3p represented the highest sensitivity for detecting EP. Combining miR-323-3p with hCG and progesterone may be useful to improve the diagnostic sensitivity in multimarker panel for the early detection of EP.

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The Role of Adrenomedullin in the Barrier of the Primary Decidual Zone during Implantation. Stephanie L Pierce,[1] Manyu Li,[1] Alan S Fanning,[1] Steven L Young,[2] Kathleen M Caron.[1] *[1]Cell and Molecular Physiology, University of North Carolina, Chapel Hill, NC, USA; [2]Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC, USA.*

The factors and mechanisms involved in protecting the embryo from maternal immune rejection during early implantation are not fully understood. Despite the presence of paternal antigens, the fetus possesses a privileged status from the maternal immune system throughout development. During early implantation, embryonic tissue is surrounded by the primary decidual zone (PDZ), which contains densely packed stromal cells with junctional proteins that likely prevent maternal immunoglobulins from reaching the embryo. However, the factors that contribute to the establishment and maintenance of these junctional proteins are not well-known. An important component of pregnancy maintenance is the small peptide adrenomedullin (AM). $AM^{+/-}$ pregnant mice, which have a 50% reduction in AM expression, show increased fetal demise and fetal growth restriction by E9.5 regardless of pup genotype. We have found that the implantation region at E5 has high AM expression, while AM is absent throughout the rest of the decidua. This suggests that AM could directly function to establish and maintain this region in order to protect early embryo development. Our lab determined that AM potently contributes to the organization of junctional proteins in endothelial cells. Thus, we hypothesize that AM functions to organize junctional proteins in the PDZ. To test this, I will use genetic mouse models with decreased AM to determine whether the reduction of this peptide can functionally diminish the barrier by using in vivo permeability assays and immunohistochemistry to assess junctional protein organization. To discern if maternal AM is responsible for the barrier function, I will eliminate the influence of fetal AM by using pseudopregnant females and a bead-induced decidualization approach for PDZ formation in WT and AM heterozygous females. Elucidation of factors that establish and maintain the barrier of the PDZ will help advance our understanding of genetic factors that may contribute to early miscarriage and intrauterine growth restriction.

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Placental Pathology of Discordant Twins with Normal and Abnormal Umbilical Artery Doppler Studies. Hilary Roeder,[1] Lynlee Wolfe,[1] Neha Trivedi,[1] Kurt Benirschke,[2] David Schrimmer.[1] *[1]Department of Reproductive Medicine, UC San Diego Health System, San Diego, CA; [2]Department of Pathology, UC San Diego Health System, San Diego, CA.*

Objective: To determine if placental pathology differs in discordant twins with abnormal compared to those with normal umbilical artery (UA) Doppler studies.

Study Design: This retrospective cohort study identified discordant twin gestations, as defined by a weight discrepancy greater than 20 percent, at our institution between 4/2003 and 6/2010. Subjects were categorized by the presence or absence of abnormal Dopplers (S/D ratio greater than 95th percentile, absent or reverse waveform) in one or both fetuses. The primary outcome was the presence of pathologic placental findings. Data abstracted included: placental weight (PW), aberrant velamentous or marginal placental cord insertion (PCI), placental evidence of fetal hypoxic stress (meconium, nucleated red blood cells), infection (chorioamnionitis, fetal vasculitis), insufficiency (infarction, hypermaturity, increased fibrin, hematoma, maternal

hypertensive changes), villitis, and intervillous thrombus. Secondary outcomes included gestational age (GA) at delivery, birthweight (BW) and BW percentile. Bivariate and regression analysis were performed.

Results: Eighty-two pregnancies were identified; 47 had normal and 35 had abnormal Dopplers. There were no differences in maternal age or chorionicity between the groups. Using logistic regression to correct for GA, PW was smaller in those with abnormal Dopplers (528±178 vs 733±149g, p<.001). Those with abnormal Dopplers were more likely to deliver earlier (32±3 vs 35±3 weeks GA, p<.001), have an abnormal PCI (49 vs 27%, p=.009), have a lower BW (1513±558 vs 2300±684g, p<.001) and BW percentile (median 9 [25th-75th percentile, 2-25] vs median 17 [25th-75th percentile, 4-32], p<.001). No significant differences in placental pathology were identified between groups with respect to fetal hypoxic stress, placental insufficiency, infection, or villitis.

Conclusions: Our results suggest that abnormal UA Dopplers in discordant twins are associated with both a decrease in placental mass and an anomalous PCI. Performing sonograms to document PCI and evaluate placental size and morphology may better identify a twin fetus significantly at risk for growth restriction, preterm birth, and subsequent perinatal morbidity and mortality.

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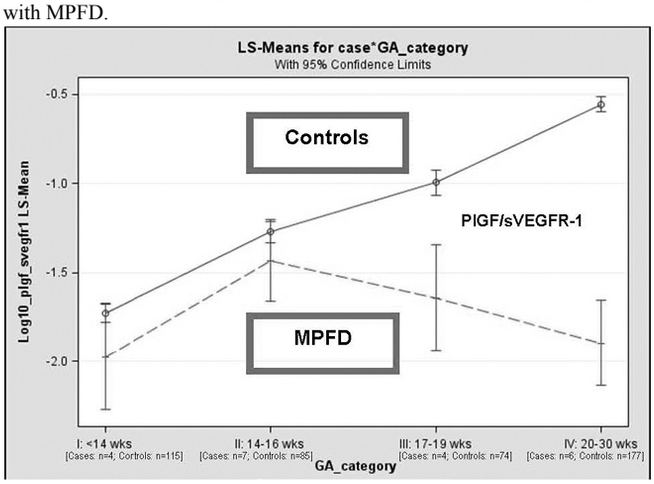
Evidence for a Role of an Imbalance of Angiogenic/Anti-Angiogenic Factors in Massive Perivillous Fibrin Deposition. Amy Whitten,[1,2] Tinnakorn Chaiworapongsa,[1,2] Roberto Romero,[1] Steven J Korzeniewski,[1,2] Adi L Tarca,[1] Eleazar Soto,[1,2] Lami Yeo,[1,2] Zhong Dong,[1] Sonia S Hassan.[1,2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective: An imbalance in angiogenic and anti-angiogenic factors have been observed in several adverse pregnancy outcomes including preeclampsia, fetal growth restriction, preterm labor, and fetal demise. Massive perivillous fibrin deposition (MPFD) is a serious complication of pregnancy associated with recurrent fetal growth restriction, second trimester spontaneous abortion, and fetal demise. The aim of the study was to determine if maternal plasma concentrations of angiogenic and anti-angiogenic factor concentrations in MPFD differ from uncomplicated pregnancies.

Methods: MPFD cases were identified by reviewing placental pathology records at our institution (n=10). Controls (n=180) consisted of patients with uncomplicated pregnancies who were enrolled in a longitudinal study and who delivered at term. Serial plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), soluble vascular endothelial growth factor receptor (sVEGFR) -1 and -2 were determined by ELISA (cases, n=31 samples; controls, n=1078 samples). Linear mixed models were used to test for differences in log transformed mean analyte concentration as a function of time adjusting for factors significantly associated with MPFD.

Results: 1) Patients with MPFD had a lower mean plasma PlGF concentration than controls (overall; p=0.004) and as a function of time (p=0.001); 2) The mean plasma concentrations of sVEGFR-1 and sEng were significantly higher in patients with MPFD than in the control group (p<0.001 and 0.003, respectively); 3) PlGF/sEng and PlGF/sVEGFR-1 ratios were lower in patients with MPFD than in the control group (p <0.001 and p<0.0001) and as a function of time (p <0.001 and p<0.0001).

Conclusion: An imbalance of angiogenic/anti-angiogenic factors is present in patients with MPFD. We propose that these changes participate in the mechanism of disease responsible for adverse pregnancy outcome in patients with MPFD.



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Uterine Artery Doppler Indices in a High-Risk Population Correlate with Pathologic Evidence of Placental Insufficiency. Lynlee Wolfe,[1] Kristen Quinn,[1] Veronique Tache,[2] Andrew Spencer,[1] Sandra Leon,[3] Kristen Klisser,[1] Louise Laurent,[1] Douglas Woelkers,[1] Mana Parast.[3] *[1]Reproductive Medicine, UC San Diego; [2]Reproductive Medicine, UC Davis; [3]Pathology, UC San Diego.*

Objective

To determine whether abnormal uterine artery Doppler indices correlate with pathologic evidence of placental insufficiency in a high-risk population.

Study Design

A retrospective analysis was conducted on patients referred to a high-risk clinic for abnormal maternal serum screen, history of adverse pregnancy outcome or preexisting medical illness. Uterine artery Doppler studies were performed between 23-27 weeks. A study was deemed abnormal if there was any diastolic notching or elevated pulsatility index (PI) ≥ 1.6. Placental pathology was performed by a single pathologist and categorized as: 1) markers of fetal hypoxic stress (meconium, nucleated red blood cells), 2) infection (chorioamnionitis, fetal vasculitis), 3) placental insufficiency (infarction, hypermaturity, increased fibrin, hematoma, maternal hypertensive changes) and 4) villitis. Clinical outcomes included placenta weight percentile (PW%), birthweight percentile (BW%), PW/BW ratio percentile and gestational age (GA) at delivery. Bivariate analysis was performed.

Results

40 pregnancies had pathologic examination of the placenta and were included. Twenty-three had normal and 17 had abnormal Doppler studies. Of the 17 patients with abnormal Doppler indices, 5 had notching alone, 5 had an elevated PI alone, and 7 had both. There was no difference in maternal age, parity, GA at delivery or umbilical cord insertion between groups. Patients with abnormal uterine artery Dopplers were more likely to have pathologic evidence of placental insufficiency (65% vs 30%; p=0.05) and a lower median BW% (18 vs 34; p=0.02). There were no differences in the rates of other types of placental pathology between the groups. Based on nomographs, 48% of subjects had PW <10th percentile and 75% had PW/BW ratio <10th percentile.

Conclusion

Among the high-risk women in this clinic, 75% had evidence of relative placental undergrowth. Even within this cohort, abnormal uterine artery Dopplers distinguished those who would develop specific pathologic evidence of placental insufficiency and low birthweight. These findings suggest the association between abnormal uterine artery Dopplers and adverse pregnancy outcomes are due, not just to placental undergrowth, but to specific pathological processes within the placenta.

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Neonatal Selective Head Cooling: Associated Placental Pathology. Corinne Yeh,[1] Poonam Khullar,[2] Michael Demishev,[1] Iman Saleh,[1] Wendy Kinzler,[1] Martin Chavez,[1] Anthony Vintzileos.[1] *[1]Obstetrics and Gynecology, Winthrop University Hospital, Mineola, NY, USA; [2]Pathology, Winthrop University Hospital, Mineola, NY, USA.*

Objective: To characterize the placental findings of neonates that underwent selective head cooling for hypoxic-ischemic encephalopathy (HIE).

Method: This is a retrospective descriptive study of placentas of neonates that underwent selective head cooling for HIE from 1/2008 to 7/2011. Placental findings were reviewed by a single perinatal pathologist. Additional data were obtained from the medical record.

Results: 9 neonates underwent selective head cooling during the study period, and placental information was available for all. The mean gestational age at delivery was 38 0/7 weeks (range 35 4/7 to 40 4/7 weeks). The mean placental weight was 424 g (range 254-584 g). Four (44%) placentas were <10th percentile for gestational age. Infarcts were seen in 2 of these; 1 also had atherosis. Retroplacental hematomas were found in 2 (22%) placentas. Severe chorioamnionitis with funisitis, chronic villitis, fetal thrombotic vasculopathy, and a large torn intramembranous vessel with hemorrhage were seen in 1 (11%) placenta each. One (11%) placenta had meconium deposition and a few increased nucleated red blood cells (nRBC). A loose, true knot without thrombi was seen in 1 (11%) placenta. Several placentas had multiple findings (Table 1).

Friday

Placental findings in neonates who underwent selective head cooling for hypoxic-ischemic encephalopathy

Subject Number	1 2 3	4 5 6[1]	7 8 9
Placental Weight < 10 percentile		X X X	X
Infarct		X	X
Atherosis		X	
Retroplacental Hematoma	X	X	
Nucleated Red Blood Cells	X		
Meconium Deposition	X		
Severe Chorioamnionitis with Funisitis			X
Chronic Villitis			X
Fetal Thrombotic Vasculopathy	X		
True Knot	X		
Torn Intramembranous Vessel, with Hemorrhage			X

[1]Uterine rupture was noted at delivery

Conclusion: Placental lesions of neonates that undergo selective head cooling for HIE are varied and include both acute and chronic processes. In our study, 11 of the 16 findings were more chronic in nature: small placenta, infarct, atherosis, increased nRBC, chronic villitis, fetal thrombotic vasculopathy, and true knot. The preponderance of lesions suggestive of chronic processes warrants further study with clinical correlation to identify antepartum factors that could be useful for assessing the risk of neonatal hypoxic-ischemic encephalopathy and provide opportunities for its prevention.

F-298

Inter-Regulation of Autophagy and NDRG1 in JEG-3 Cells under Hypoxic Condition. Hyang Gi Park, Ji Yeon You, Suk-Joo Choi, Soo-young Oh, Jong-Hwa Kim, Cheong-Rae Roh. *Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.*

Objective: N-myc downstream-regulated gene 1 (NDRG1) modulates the response of trophoblasts to hypoxia and promotes cell survival. Autophagy is an intracellular bulk degradation system responsible for lysosomal degradation of protein and other subcellular constituents. In this study, we investigated inter-regulation between autophagy and NDRG1 in JEG-3 cells under hypoxic condition.

Methods: To inhibit the autophagic activity of JEG-3 cells *in vitro*, we transfected JEG-3 cells with siRNA for LC3. Up- and down-regulation of NDRG1 was induced by transfection of NDRG1 plasmid DNA and siRNA for NDRG1, respectively. Expression of LC3-II and NDRG1 were assessed by immunoblotting. Inter-regulation between autophagy and NDRG1 in JEG-3 cells were examined under $CoCl_2$ stimulated condition.

Results: As expected, transfection of each vector up- and down-regulated LC3-II and NDRG1 in JEG-3 cells, respectively. Under $CoCl_2$ stimulated condition, the expression of NDRG1 increased significantly, however, the expression of LC3-II did not change. Inhibition of autophagy did not induce any change in the level of NDRG1 expression under $CoCl_2$ stimulated condition. Up- or down-regulation of NDRG1 did not induce any change in the level of LC3-II.

Conclusion: Modulation of NDRG1 did not affect autophagic activity, vice versa, in JEG-3 cells under hypoxic condition.

F-298-2

Increased Level of ERK1/2 Activation in Placentas of Pregnancies Complicated by Severe Intrauterine Growth Restriction. Eli Rimon,[1] Joseph B Lessing,[1] Avraham Amsterdam.[2] *[1]Obstetrics and Gynecology, Tel Aviv Medical Center, Tel Aviv University, Tel Aviv, Israel; [2]Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel.*

Introduction: The extracellular signal-regulated kinase 1 and 2 (ERK1/2) play a central role in trophoblasts differentiation. ERK1/2 are cytoplasmatic proteins in resting cells and translocate to the nucleus upon stimulation as the activated form-phosphorylated ERK. It has been shown that hypoxia influences trophoblasts differentiation in both in-vivo and in-vitro models. Therefore, we hypothesized that ERK 1/2 activation might be affected in placentas obtained from women with severe intrauterine growth restriction (IUGR), a pregnancy complication associated with placental hypoxia.

Objective: to investigate the activation of ERK1/2 in placentas of women with severe IUGR.

Methods: Tissue samples were collected from placentas of women with severe IUGR (8 women), defined as birth weight below 5th percentile for Israeli population, and women with normal pregnancy and delivery (10 women). The placental samples were immunostained for phosphorylated ERK1/2 .For scoring the number of labeled nuclei pictures were taken at X 1000 magnification. Three different slides from each placenta were photographed and at least three different areas in each slide were chosen randomly. We counted the number of labeled nuclei out of the total number of nuclei in the syncytiotrophoblast layer and calculated the percentage of labeled nuclei in each photograph. Total number of 5000 nuclei were investigated in both groups. Student t- test was used to compare the mean±SD in the IUGR and normal groups. Importantly, we confirmed the presence of phosphorylated ERK in the nuclei of syncytiotrophoblast by electron microscopy.

Results: 52% (±14.7) of the nuclei in the syncytiotrophoblast nuclei were labeled in normal placentas compared to 78.9 % (±6.9) in IUGR placentas ($p<0.05$).

Conclusions: We have shown a higher rate of ERK1/2 activation, expressed by presence of phosphorylated ERK1/2 in the nuclei, in placentas of pregnancies complicated by severe IUGR. Since inhibitors of ERK1/2 impair trophoblasts differentiation, increased activation of ERK1/2 (i.e. growth factors signal) may represent a mechanism by which trophoblasts preserve their function in continuous hypoxia.

F-299

Monocyte Subpopulations in Pregnancy Complicated by Pre-Eclampsia Demonstrate a Pro-Inflammatory Phenotype and Altered Angiogenesis, Chemotaxis and Migration. Ebtisam A Al-ofi,[1] Seth Coffelt,[2] Dilly Anumba.[1] *[1]Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield Medical School, Sheffield, United Kingdom; [2]Division of Molecular Biology, de Visser Lab, The Netherlands Cancer Institute/Antoni Van, Amsterdam, Netherlands.*

Preeclampsia (PE) is associated with an exaggerated systemic inflammatory response (ESIR). We have demonstrated that monocyte (Mo) subtypes in PE demonstrate a pro-inflammatory phenotype and altered functional expression of Toll like receptors 2 and 4. We postulated that ESIR in PE may be triggered by circulating endogenous ligand(s) of TLR2 and 4, and sought to investigate the expression of markers of angiogenesis, chemotaxis and migration in peripheral blood mononuclear cells (PBMCs) isolated from normal pregnant (NP, n=15), PE (n=15), and non pregnant (n=10) women. PBMCs were stained with antibodies to TLR2, TLR4, CD14, CD16, HLA-DR (a MHC class II cell surface receptor) or isotypes, and also labelled with antibodies to Tie2 (a receptor of angiopoietin), CCR2 (a receptor of the monocyte chemo-attractant protein-1), and CCR5 (a receptor of the macrophage inflammatory proteins) or isotypes. PBMCs were then analysed by flow cytometry. Plasma levels of endogenous TLR4 ligands- heparan sulfate, hyaluronan, fibronectin, fibrinogen and High mobility group box-1(HMGB1) - were measured by ELISA. Mo was stimulated by LPS, peptidoglycan and endogenous ligands, and cytokine levels measured by cytometric array. Compared to NP, the proportion of monocytes with the CD14+CD16+ phenotype was higher in PE ($P<0.001$) while CD14++CD16- was lower, and TLR2 and 4 expressions were higher ($P<0.001$), and differed between monocyte subpopulations. Compared to NP, HLA-DR and Tie2 expressions were lower in PE ($P<0.01$), whilst CCR2 and 5 were higher ($P<0.01$). Plasma levels of heparan sulfate, hyaluronan, fibronectin and fibrinogen did not differ, but HMGB1 was higher in PE ($P<0.05$). In pre-eclampsia, stimulation of monocytes with peptidoglycan, E coli LPS and fibrinogen was associated with an exaggerated release of TNF-α and IL-6, consistent with ESIR. Upregulation of TLR-2 and 4, downregulation of the angiogenic marker Tie 2, and upregulation of the migration factors CCR2 and 5 are likely to be key modulatory events in the pathogenesis of the vascular injury seen in preeclampsia.

F-300

First Trimester Placental Gene Expression Analysis in Patients with Elevated Serum Levels of Free Fetal Hemoglobin and Alpha-1-Microglobulin. Ulrik D Anderson,[1] Vera Casslen,[1] Bo Akerstrom,[2] Judith Cartwright,[3] Baskaran Thilaganahtan,[3] Stefan R Hansson.[1] *[1]Obstetrics and Gynecology, University Hospital of Skåne in Lund, Lund, Sweden; [2]Dept. of Clinical Sciences, University of Lund, Lund, Sweden; [3]Academic Department of Obstetrics and Gynecology, St. Georges University Hospital, London, United Kingdom.*

Background: Gene expression studies have shown up-regulation of genes coding for hemoglobin in term preeclamptic (PE) placentas. Free fetal hemoglobin (HbF) measured in maternal serum has been shown to be a sensitive and specific first trimester biomarker of subsequent development of PE. First trimester Doppler ultrasound (RI) of the uterine arteries can identify patients at high risk of developing PE. The aim of this study was to perform gene profiling of placentas sampled in the first trimester from patients with elevated serum HbF levels.

Materials and methods: 15 placenta samples were collected from pregnancies terminated at 9-11 weeks of gestation. 8 high-resistance cases were identified based on Doppler ultrasound of the uterine arteries. Maternal serum was

analyzed for HbF with ELISA and the heme scavenger α-1-microglobulin (A1M) with RIA. Total mRNA was isolated from the placenta samples. Whole genome microarray was done using the Illumina platform provided by SCIBLU Genomics, Lund University using the HumanHT-12 v4 Expression BeadChip. Statistics was done with MeV software. Bioinformatics was performed with DAVID 6.7. Analysis was performed blinded for the patient categories.
Results: Serum analysis for HbF and A1M showed significant differences between the groups. The pregnancies with normal RI showed elevated HbF and A1M serum levels compared to the high RI group. Bioinformatics showed significant alterations in the gene expression for the Gene Ontology terms: Pore complex biogenesis, Negative regulation of smooth muscle contraction, Negative regulation of inflammatory response, Positive regulation of adaptive immune response and the MAPK pathway.
Conclusions: The gene expression analysis showed disturbances of important pathways in patients with elevated serum level of HbF. Several of the dys-regulated genes seems relevant for development of PE. We found genes that might increase the blood flow to the placenta or modulate the immune system to reduce the inflammatory response seen in PE. This might reflect protective mechanisms. A better understanding of the protective mechanisms may provide new ideas for future therapeutics.

F-301

New Target Pathways for Preeclampsia: Differential Methylation of Genes Associated with Cell Adhesion in Preeclamptic Placentas. Lauren Anton, Michal A Elovitz. *Obstetrics and Gynecology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA.*
Introduction: Preeclampsia (PRE) is associated with abnormal expression of factors that influence placental growth and function. The mechanisms that govern these changes in gene expression are unknown. We investigated if alterations in gene specific DNA methylation, specifically genes that regulate placental function, contribute to PRE development.
Methods: Placental biopsies were collected from normal pregnant women delivering at term (n=14), with term PRE (≥37 weeks; n=19) or with preterm PRE (<37 weeks, n=13) and DNA and RNA were extracted. The DNA underwent bisulfite modification and hybridization to the Illumina Methylation 450 BeadChip. Probe specific analysis was performed using a 5% methylation difference cutoff and a false negative p value <0.05. Differentially methylated genes clustering to significant functional pathways were identified. Expression of a subset of genes was investigated in PRE and control placentas by QPCR. To confirm methylation effects in trophoblasts, RNA was extracted from first trimester extravillous trophoblasts (EVTs) treated with demethylating agent 5-aza-2'-deoxycytidine (5 umol/L) (n=6) or control (n=6) for 4 days.
Results: Of the 485,582 gene loci on the array, compared to controls, 154 genes were differentially methylated in PRE placentas, 33 in term PRE and 1448 genes in preterm PRE. Functional annotation of the differentially methylated genes in preterm PRE placentas revealed a 32 gene cluster in the cell adhesion functional group ($p<0.0001$). CDH11 ($p<0.05$), COL5A1 ($p<0.01$), NCAM1 ($p<0.05$) and TNF ($p<0.05$) mRNA expression was altered in preterm PRE placentas by QPCR. These results were validated in demethylated EVTs with alterations in CDH11 ($p<0.05$), COL5A1 ($p<0.05$), NCAM1 ($p<0.05$) and TNF ($p<0.001$) mRNA expression.
Conclusion: These studies demonstrate that differential methylation results in changes in placental mRNA expression suggesting that epigenetic regulation of these genes contributes to placental dysfunction and/or disease development. The increase in differentially methylated genes in preterm PRE placentas suggests that altered gene methylation correlates with disease severity. Furthermore, we provide evidence that disruption of methylation in first trimester trophoblasts alters expression of these genes demonstrating that epigenetic modifications early in pregnancy can have effects on trophoblast function contributing to PRE.

F-302

Maternal Cardiac Dysfunction in a Mouse Model of Preeclampsia Induced by Overexpression of sFlt-1. Martina T Ayad, Julio F Mateus, Kathleen L Vincent, Jinping Yang, Esther H Tamayo, George R Saade. *OB/GYN, University of Texas Medical Branch, Galveston, TX, USA.*
Objective: We have previously demonstrated that pregnant mice overexpressing sFlt-1 develop hypertension and endothelial dysfunction. Our objective was to determine the maternal cardiac function profile in this animal model of preeclampsia using microultrasonography.
Method: Pregnant CD-1 mice underwent echocardiography using a RMV 740 scan (Visualsonics, Vevo 770) at days 8, 14, and 17 of gestation. After recovery from initial sonographic exam (day 8), mice were randomly allocated to tail vein injections with Adv-sFlt-1 (10^9 PFU) or mFc (10^9 PFU, used as control of the virus) (n = 5-6 per group). Echocardiography was performed in an anesthetized animal using 2% isoflurane in oxygen delivered by face mask. Exam was limited to 30 min per animal. M-mode, B-mode, and pulsed Doppler were used for cardiac evaluation. Plasma levels of sFlt-1 were measured at days 8, 14, and 18 of gestation. Animals were sacrificed at day 18 of gestation. One-way ANOVA was used for statistical analysis (significance: $P < .05$).
Results: A significant increase in the left ventricle diastolic and systolic inner diameters (LVDID and LVSID) was seen in the sFlt-1 compared with the mFc group (delta = + .56 mm and + .67 mm vs -.12 mm and -.33 mm, respectively; $P < .05$) between days 8 and 14 of gestation. A similar pattern was observed with the left ventricle diastolic and systolic volumes (LVDV and LVSV) among the sFlt-1 and mFc groups (delta = + 21.24 µl and + 14.85 µl vs – 5.44 µl and – 7.99 µl; $P < .05$). During this interval, LV ejection fraction (LVEF) was reduced by 11% in the sFlt-1 group, while it increased 5% in the mFc group ($P < .05$). Between days 14 and 17 of gestation, there was an increase in the LVDID and, LVSID (delta = + .33 and + .24 mm) as well as in the LVDV and LVSV (delta = + 13.27 µl and 5.65 µl) in the mFc mice, while these parameters decreased in the sFlt-1 group (LVDID and LVSID delta = - .17 mm and -.34 mm; LVDV and LVSV delta = -8.56 µl and 9.56 µl). At this interval, LVEF increased by 9% in the sFlt-1 mice and it increased 1% in the mFc group ($P < .05$).
Conclusion: Overexpression of sFlt-1 resulted in maternal left ventricular systolic dysfunction in the mid gestation. Recovery of cardiac function in late gestation may be attributable to compensatory mechanisms.

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Placental Glucose Transporter 9 in Pre-Eclampsia. Florence Tschopp,[1] Daniel V Surbek,[1] Marianne Messerli,[1] Christiane Albrecht,[2] Matthias Hediger,[2] Marc U Baumann.[1] *[1]Obstetrics and Gynecology, University Hospital of Berne, Berne, Switzerland; [2]Institute for Biochemistry and Molecular Medicine, University of Berne, Berne, Switzerland.*
OBJECTIVES: Glucose transporter (GLUT) 9, a member of the GLUT family, is a high-capacity transporter of uric acid and was shown to be expressed in various tissues. Uric acid seems to be involved in the pathogenesis of pre-eclampsia (PE). Further, uric serum levels correlate with fetal outcome. We have previously detected GLUT9 mRNA levels in villous tissue. We hypothesized that the uric acid transport system and its transporter GLUT9 were altered in PE. The aim of this study was to analyze placental GLUT9 expression following normal pregnancies and pregnancies compromised by PE.
METHODS: Placental villous tissue was homogenized following Ceasarean sections after normal pregnancies (n=4) and pregnancies with PE (n=4). Western blottings were performed and GLUT9 protein expression was assessed by an immunoanalytical assay using specific antibodies.
RESULTS: GLUT9 protein was detected in villous tissue. Compared to controls GLUT9 expression levels in villous tissue were not different in PE.
CONCLUSIONS: These preliminary data indicate that the placental GLUT9 expression is not altered in PE when compared to control. Ongoing studies will determine whether in PE GLUT9 shows an alteration of the expression pattern in syncytial membrane fractions (basal membrane and microvillous (apical) membrane fractions) allowing to clarify the role of GLUT9 in PE. This might enable to elaborate preventive strategies for pregnancy-specific diseases such as PE.
This study is supported by the Swiss National Fundation (SNF) project TRANSCURE.

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Angiogenic Factor Imbalance Is Not Specific to the Maternal Syndrome of Pre-Eclampsia. Samantha Benton,[1] Ulla Knudsen,[3] Yuxiang Hu,[1] Erik Vittinghus,[3] Jim Allen,[3] Camilla Konborg,[3] Kenneth Kupfer,[4] Xie Fang,[1] Laura Magee,[2] Peter von Dadelszan.[1] *[1]Ob/Gyn, UBC, Vancouver, BC, Canada; [2]Medicine, UBC, Vancouver, BC, Canada; [3]Ob/Gyn, Randers Hospital, Denmark; [4]Alere, San Diego, CA, USA.*
Objective: It has been proposed that angiogenic factor imbalance directly causes the maternal syndrome of pre-eclampsia (PET). Therefore, this imbalance should not be seen in women with fetal growth restriction resulting from placental dysfunction (placental intrauterine growth restriction (IUGR)). Our hypothesis is that changes in angiogenic factors reflect the underlying placental pathology of both early-onset (EO) PET and placental IUGR and will not discriminate between them.
Methods: Plasma was collected from 25 women with EO PET (≤35wk), 19 with late-onset (LO) PET (>35wk) and 9 normotensive women with placental

IUGR (fetal abdominal circumference <10th centile for gestational age (GA) on ultrasound, confirmed by birthweight <10th centile and abnormal placental pathology). Women with uncomplicated pregnancies served as controls (n=79). Analytes were quantified in batch assay using the Triage immuoassay (placental growth factor [PlGF], soluble endoglin [Eng]) and conventional ELISA (soluble Fms-like tyrosine kinase-1 [sFlt-1]) (Alere, San Diego). Power to discriminate between EO PET and placental IUGR was assessed for each analyte by comparing true positive test results in each group. A positive result was defined as a concentration <5th centile (PlGF) or >95th centile (Eng, sFlt-1) for GA in normal pregnancy.

Results: Concentrations of PlGF were decreased in EO PET and placental IUGR while Eng and sFlt-1 were increased. Positive test results were more common in EO PET and placental IUGR but test performance for any analyte did not differ significantly (Fisher's exact p>0.05).

Prevalence of positive test results in study groups.

	Early-onset PET, n=25	Late-onset PET, n=19	Placental IUGR, n=9	Uncomplicated pregnancy, n=79
PlGF	25 (100%)	9 (47%)	9 (100%)	4 (5%)
Eng	11 (48%) (n=23)	4 (21%)	6 (67%)	1 (1%) (n=78)
sFlt-1	18 (72%)	4 (21%)	5 (56%)	1 (1%)

Conclusion: In this preliminary study, angiogenic factors do not appear to discriminate between EO PET and placental IUGR. Trends in angiogenic imbalance in PET and placental IUGR appear to reflect underling placental pathology and are not causally-related to the pathogenesis of the maternal syndrome of PET. Results are currently being confirmed in a larger cohort of women with PET and placental IUGR.

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Arterial Compliance Prior to and during Pregnancy and Subsequent Risk for Preeclampsia. Ira M Bernstein,[1] Sarah Hale,[1] Gary J Badger.[2] [1]*Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA;* [2]*Medical Biostatistics, University of Vermont.*

OBJECTIVE: We have hypothesized that reduced prepregnancy arterial compliance contributes to the new development of hypertension in pregnancy in response to volume expansion. In this study we prospectively examined pulse wave velocity (PWV), an index of arterial compliance and an established risk factor for hypertension both prior to, and during, pregnancy to evaluate it as a risk for the development of preeclampsia.

STUDY DESIGN: Twenty young healthy nulliparous subjects were enrolled in a longitudinal study. All subjects achieved singleton pregnancies and delivered at term. Seventeen of the 20 pregnancies were normal (CTL) and 3 developed preeclampsia (PRE). Women were evaluated prior to pregnancy during the follicular phase of the menstrual cycle (PP), in early pregnancy (EP, 11-14 weeks) and during late pregnancy (LP, 30-34 weeks). We compared prepregnancy, EP and LP PWV between those who subsequently developed preeclampsia and those who did not. PWV was measured using simultaneous electrocardiographic tracings and ultrasound determined arterial flow waveforms and calculated as the estimated distance divided by interval between EKG r-wave peak and ultrasound derived peak brachial artery flow. Data are expressed as mean ± SE. $P < 0.05$ accepted for significance.

RESULTS: We observed no significant differences in maternal age, BMI or supine mean arterial pressure PP comparing PRE and CTL. We identified a significant increase in prepregnancy PWV in subjects destined to develop preeclampsia (PRE: 2.99 ± 0.13, CTL: 2.66 ± 0.05 m/s, P=0.02). This difference was not evident at either of the pregnancy time points (EP: PRE: 2.90 ± 0.20, CTL: 2.72 ± 0.05 m/s, p = 0.21; LP: PRE: 2.61 ± 0.06, CTL: 2.57 ± 0.06 m/s, p = 0.76).

CONCLUSION: In this small prospective longitudinal study arterial compliance, as estimated by PWV and evaluated prior to pregnancy, is strongly associated with the subsequent development of preeclampsia. Further studies to clarify the relationship of prepregnancy arterial compliance to the subsequent development of preeclampsia are ongoing.

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GSK3β Reduces Mcl-1 Phosphorylation in Preeclamptic Placentae. Jayonta Bhattacharjee, Isabella Caniggia. *Dept. of Ob./Gyn., SLRI, Mount Sinai Hospital; University of Toronto.*

Introduction: Myeloid cell leukaemia factor 1(Mcl-1) is an anti-apoptotic Bcl-2 family member that plays a key role as a regulator of trophoblast cell fate. Mcl-1 is a short lived protein whose stability is tightly regulated through post-translational modifications including proteolytic caspase cleavage and splicing generating pro-apoptotic products. Mcl-1 protein stability is also controlled by phosphorylation via GSK3β leading to Mcl-1 proteosomal degradation. However, when GSK3β itself gets phosphorylated, it becomes inactive and does not phosphorylate Mcl-1. We have previously reported an altered Mcl-1 expression in preeclampsia (PE) and have shown that this is in part due to active caspase cleavage of Mcl-1 following oxidative stress. Herein, we examined the phosporylation of of Mcl-1 (pMcl-1) and GSK3b in placentae from normotensive and preeclamptic pregnancies. **Methods:** Placental tissues from severe early onset PE (n=17), and preterm aged-matched control (AMC) (n=9) were used. pMcl-1, total and phospho-GSK3β protein levels were measured by immunoblotting. Mcl-1/GSK3β association was assessed by immunoprecipatation. The effect of oxidative stress and hypoxia on Mcl-1 phosphorylation and total and phospho-GSK3b was assessed by treating choriocarcinoma JEG3 cells with, sodium nitroprosside (SNP; 2.5 mM and 5.0 mM), a nitric oxide donor, and 3% O_2 with or without proteosomal inhibitor MG132. **Results:** Phospho-Mcl-1and total GSK3b protein levels were significantly ($p<0.05$) reduced in preeclampsia relative to controls, while, notably, phospho-GSK3b levels were markedly increased. Similarly, following SNP and 3% O_2 treatments, GSK3β and phospho-Mcl-1 were decreased and this associated with increased expression of phospho-GSK3b. Immunoprecipitation experiments revealed a decreased Mcl-1/GSK3β association in preeclamptic placenta compared to AMC. When cells were treated with a specific GSK3β phosphorylation inhibitor (GSK-3 Inhibitor IX), phospho-Mcl-1 levels significantly decreased suggesting a contribution for GSK3β in Mcl-1 phosphorylation in trophoblast cells. **Conclusion:** Oxidative stress-induced phosphorylation of GSK3b is in part responsible for a reduction in Mcl-1 phosphorylation in preeclamptic placentae thereby contributing to the altered Mcl-1 expression in this disease. (Supported by CHIR and Canada-Hope Fellowship)

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Novel Role of Pro-Survival Mcl-1L in Trophoblast Cell Proliferation in Human Placental Development and in Preeclampsia. Jayonta Bhattacharjee, Alessandro Rolfo, Isabella Caniggia. *Dept. of Ob./Gyn., SLRI, Mount Sinai Hospital; University of Toronto, ON, Canada.*

Mcl-1L is a pro survival Bcl-2 family member that plays a key role in shaping placental development by regulating trophoblast cell fate. In oxidative stress condition, Mcl-1L undergoes cleavage and splicing generating pro-apoptotic Mcl-1c and Mcl-1S products. Another Mcl-1 variant termed small nuclear Mcl-1 (snMcl-1) inhibits cell cycle progression by interacting with cell-cycle-dependent kinase CDK-1 thus preventing its binding to Cyclin B1. However, the role of Mcl-1 isoforms in proliferation in the human placenta remains to be established. Herein, we investigated the involvement of Mcl-1 in trophoblast cell proliferation in human placental development and in preeclampsia (PE). Placenta tissues (5-15 weeks, n=21) throughout gestation and from PE (n=24) and age matched controls (AMC, n=14) pregnancies were used. Protein levels were measured by immunoblotting using CDK-1, Cyclin B1 and Mcl-1 antibodies. CDK-1/Mcl-1, CDK-1/Cyclin B1 association and localization were assessed by immunofluorescence (IF). In order to establish the role of Mcl-1 in cellular proliferation, choriocarcinoma JEG3cells were transfected with different Mcl-1 constructs (Mcl-1L and Mcl-1c) and proliferation was assessed by immunoblotting for PCNA. CDK-1 and Cyclin B1 proteins showed a peak of expression at 5-8 weeks of gestation that inversely correlated to that of Mcl-1. IF revealed strong positive CDK-1 and Cyclin B1 signals in the cytotrophoblastic nuclei during early gestation, while Mcl-1 was mainly localized to the cytoplasm of syncytiotrophoblast cells. With advancing gestation (9-15 weeks), CDK-1/Mcl-1 co-localization was found in the nuclei of cytotrophoblast and extravillous trophoblast cells. Notably, Mcl-1/CDK-1 co-localization was observed in PE trophoblast cells relative to AMC. Overexpression of JEG3 cells with Mcl-1L, but not Mcl-1c, resulted in a significant reduction of the proliferation marker PCNA. During early placentation high CDK-1/Cyclin B1 levels found in trophoblast cells may account for increased trophoblast proliferation rates; while, with advancing gestation, increased CDK-1/Mcl-1 nuclear association implicates a novel role for Mcl-1L as an inhibitor of trophoblast cell cycle progression. In preeclampsia, increased CDK-1/Mcl-1 association may contribute to altered trophoblast proliferation typical of this pathology. (Supported by CIHR and Canada-Hope Fellowship)

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Preeclampsia Is Characterized by Placental Complement Dysregulation. Aletta Buurma,[1] Danielle Cohen,[1] Frans Claas,[2] Jan Anthonie Bruijn,[1] Kitty Bloemenkamp,[3] Hans Baelde.[1] *[1]Dept. of Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Dept. of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands; [3]Dept. of Obstetrics, Leiden University Medical Center, Leiden, Netherlands.*

Introduction

Increasing evidence suggests that preeclampsia (PE) is associated with complement dysregulation. Mutations in complement regulatory proteins predispose to PE and women with PE have increased circulating complement activation products compared to healthy subjects. The origin of complement dysregulation is unknown, and further unraveling this mechanism could provide important therapeutic targets. We therefore investigated preeclamptic and control placentas for the presence of complement activation products and alterations in the expression of complement regulatory proteins.

Methods

Placentas from 28 women with PE (defined according to ACOG criteria) were compared to 30 placentas obtained from healthy controls. Immunohistochemical staining was performed for components specific for the three different complement activation pathways; C1q, MBL, properdin. Besides, we stained for C4d, a stable marker for classical and lectin pathway activation. Quantitative PCR (qPCR) was performed to measure mRNA expression of the complement regulatory proteins MCP, DAF and CD59.

Results

In both cases and controls, C1q was widely present on the fetal-maternal interface, which is in accordance with previous literature. MBL was always absent, whereas properdin was occasionally observed but never on the fetal-maternal interface. C4d was seldomly present in control placentas (3%), whereas it was observed in 14 out of 28 (50%) placentas obtained from women with PE ($p<0.01$). In these placentas, C4d observed in a focal (9/14) or diffuse (5/14) staining pattern on the fetal-maternal interface.

qPCR revealed that MCP levels were unchanged, whereas a significant upregulation was observed in the placental mRNA expression of DAF ($p<0.05$) and CD59 ($p<0.01$) in cases compared to controls.

Discussion

Based on C4d staining, there is evidence for classical complement pathway activation in placentas from women with PE compared to controls, which is suggestive for the presence of circulating (auto-) antibodies. Additionally, there is an upregulation of complement regulatory proteins, possibly providing a feedback mechanism to prevent complement mediated injury. Complement inhibition may be a novel approach to treat PE.

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The Influence of Pregnancy and Sympathetic Innervation on Cerebral Blood Flow Autoregulation during Acute Hypertension. Siu Lung Chan, Marilyn J Cipolla. *Neurology, Ob/Gyn & Repro Sci, University of Vermont, Burlington, VT, USA.*

Objective: Edema formation during eclampsia occurs preferentially in the posterior brain region for unknown reasons. One possibility is that cerebral blood flow autoregulation (CBFAR) is less effective in the posterior vs. anterior brain region. Thus, we compared CBFAR between anterior and posterior brain regions in nonpregnant (NP) and late-pregnant (LP) rats during an acute elevation in pressure. We further investigated whether decreased sympathetic innervation of posterior cerebral arteries (PCA) promotes autoregulatory breakthrough at lower pressures by measuring perivascular sympathetic nerve density of PCA vs. middle cerebral arteries (MCA) in NP and LP rats.

Methods: Female SD NP and LP (E19-21) rats (n=8/group) were anesthetized with chloral hydrate and mechanically ventilated. Laser Doppler probes were placed over the anterior and posterior brain regions and CBF measured simultaneously. Changes in CBF were recorded as blood pressure was increased from 100 and 180 mmHg by infusion of phenylephrine (1-48 μg/ml). Water content of the anterior and posterior brain regions was compared by wet:dry weights. In separate animals, PCA and MCA were dissected and stained for tyrosine hydroxylase (TH) and nerve density determined by morphometric analysis.

Results: In LP animals, CBFAR was less effective in the posterior vs. anterior region. The pressure at which CBF increased significantly from baseline was 160 mmHg in posterior vs. 170mmHg in anterior ($p<0.05$). There was no difference in CBFAR between regions in NP rats. However, in both anterior and posterior brain regions, pregnancy improved CBFAR vs. NP. The pressure at which CBF significantly increased in anterior and posterior regions was 170 and 160mmHg for LP vs. 140mmHg for NP in both regions ($p<0.05$). Brain water content was significantly increased in LP vs. NP animals for both anterior (79.28 ± 0.25 vs. $78.62 \pm 0.09\%$; $p<0.05$) and posterior (78.26 ± 0.17 vs. $77.16 \pm 0.11\%$; $p<0.05$) regions. TH nerve density was increased 2-fold in PCA vs. MCA in both LP (6.08 ± 0.97 vs. 3.49 ± 0.30 nerves/μm^2; $p<0.01$) and NP (5.45 ± 0.44 vs. 3.45 ± 0.28 nerves/μm^2; $p<0.01$) rats. There was no difference in nerve density with pregnancy.

Conclusion: Diminished autoregulation in the posterior brain during pregnancy does not appear to be related to decreased sympathetic innervation since TH nerve density was increased in PCA vs. MCA and not changed with pregnancy.

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The Genetics of the Alternative Pathway of Complement in the Pathogenesis of HELLP Syndrome. Francesca Crovetto,[1] Nicolo Borsa,[2] Richard JH Smith,[3] Flora Payvandi,[4] Edgardo Somigliana,[1] Paola Vigano,[5] Gianluigi Ardissino,[6] Barbara Acaia.[1] *[1]Obstet-Gynecol, Fondazione Cà Granda; [2]Lab Medical Genetics, Fondazione Cà Granda; [3]Nephrology, Carver College of Medicine, University of Iowa; [4]Hemophilia and Thrombosis Center, Fondazione Cà Granda; [5]Obstet-Gynecol, San Raffaele Scientific Institute; [6]Pediatric Nephrology, Fondazione Cà Granda.*

Gene mutations in the complement alternative pathway play a critical role in the pathogenesis of Atypical Hemolytic Uremic Syndrome, a thrombotic microangiopathy sharing several features with HELLP syndrome. Interestingly, Fakhouri et al. (2008) recently identified these mutations also in 4 out of 11 women with HELLP syndrome. To further investigate this issue, we screened 33 women who developed the disease for variants in alternative pathway genes. The coding sequences and intron-exon boundaries of the complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP), complement factor B (CFB) and C3 were bidirectionally sequenced on an ABI PRISM 3130 xl Genetic Analyzer. The potential association of identified variants was evaluated through literature review and computationally using the following three softwares: the PolyPhen, the Sorting Intolerant From Tolerant (SIFT) and the Mutation Taster.

We identified three women carrying genetic variants. One of them (p.Gly261Asp in exon 6 of CFI) was previously classified as a benign. The other two substitutions (p.Ser13Phe in exon 1 of MCP and p.Pro402Ser in exon 11 of CFI) were not described yet. The first was scored as benign with PolyPhen and as affecting/might affect the protein function with SIFT and Mutation Taster. The second was scored as benign/tolerated by the first two programs while Mutation Taster scored the protein function as might be affected. Overall, two women (6%, 95%CI: 1-18%) carried mutations in alternative pathway genes that may affect protein function. This frequency is lower than the 36% rate reported by Fakhouri et al. We speculate that this conflicting result may be due to a selection bias, because these authors recruited women in a nephrology center, thereby presumably selecting those with severe renal involvement.

In conclusion, the genetics of the alternative pathway of complement does not play a major role in the pathogenesis of HELLP syndrome. However, it may have a role in the subgroup of women with severe renal involvement.

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Lipidomic Analysis of Women with Early-Onset Preeclampsia. Leandro G De Oliveira,[1,2,3] Niels O Camara,[2] Nelson Sass,[1,3] Tatiana Bonetti,[1] Antonio F Moron,[1] Henri Korkes,[1] Ismael D Da Silva.[1] *[1]Obstetrics, Federal University of São Paulo, São Paulo, Brazil; [2]Imunology, University of São Paulo, São Paulo, Brazil; [3]Obstetrics, Scholl Maternity Vila Nova Cachoeirinha, São Paulo, Brazil.*

Background: preeclampsia is characterized by intense inflammatory response and an anti-angiogenic state. Maternal obesity has been considered to have important impact on the genesis of preeclampsia as lipotoxicity leads to maternal endothelial dysfunction. Here we investigate the plasma lipid profile of preeclamptic women with confirmed anti-angiogenic state. **Methods:** this study included 8 pregnant women with early-onset preeclampsia (before 34 weeks gestation) and 8 normal pregnant women. Each patient in the preeclampsia group was matched to a patient in the control group according to gestational age at the time of sample collection. All patients in the control group were followed until term and had normal babies. To characterize the anti-angiogenic state of the patients, serum concentration of sFlt-1 and PlGF were measured by automated electrochemiluminescence immunoassay (Roche Diagnostics, IN). Differences between results of sFlt-1 and PlGF were evaluated using Mann-Whitney *U* test. To investigate the lipid profile, lipids were extracted from plasma samples using the Bligh-Dyer protocol and the extracts were subjected to MALDI-TOF Mass Spectrometry. Data matrix was exported for partial least squares discriminant analysis. All the variables analysed were

sorted by a score number named Variable Importance in the Projection. The major discriminant variables were selected and underwent to Mann–Whitney U test. **Results:** the mean serum concentration of sFlt-1 was 14231 pg/mL (6609 – 18143 pg/mL) in women with preeclampsia, as compared with 1507 pg/mL (951.8 – 2242 pg/mL) in controls (P=0.0002). The mean serum concentration of PlGF was 79.37 pg/mL (31.61 – 239.0 pg/mL) in the preeclampsia group and 463.7 pg/mL (101.3 – 1987 pg/mL) in controls (P=0.0019). A total of 1290 ions were initially identified during lipidomic assessment. Twelve m/z signals were highlighted as the most important lipids for the discrimination of patients with preeclampsia. The identification of these differential lipids was carried out through Lipid Database Search. The main classes identified were Glycerophosphocholines, Glycerophosphoserines, Glycerophosphoglicerols and Glycosyldiradylglycerols. **Conclusion:** Our results suggest some lipid species as potential biomarkers for early-onset preeclampsia.

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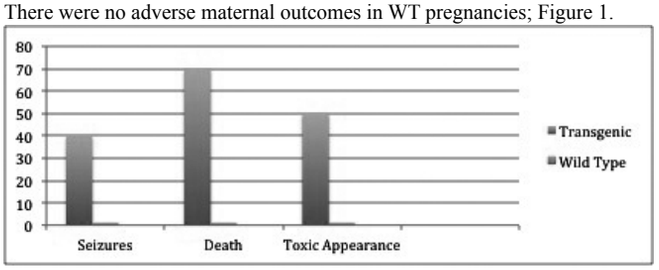
Adverse Maternal Outcome in Transgenic Mice: Potential Interspecies RAS Gene Interactions. Jeff M Denney, Cynthia E Shaw, Annette Gendron, Dinesh M Shah. *Maternal-Fetal Medicine, Perinatal Research, and Comparative Pathology, University of Wisconsin, Madison, WI, USA.*

Objective: The renin-angiotensin system (RAS) plays a key role in the regulation of blood flow and pressure. RAS has been implicated in pathophysiology of hypertensive disorders of pregnancy. Previous in vitro studies have indicated that there are no interactions between human RAS genes and mouse RAS genes due to species specificity of biological actions. We compared maternal outcome in wild type (WT) mouse gestation to mouse gestation in females transgenic (TG) for either human angiotensinogen (hAng) or human renin (hRen).

Methods: Mouse strains were genotyped for verification. Colony lines of hAng, hRen, and WT were maintained separately for our breeding protocol in this analysis to avoid interaction of the hRen with hAng transgenes in vivo. Trained clinicians and scientists assessed maternal health. Adverse maternal outcomes assessed included seizures, death, and toxic appearance. Fisher's Exact (FE) and Wilcoxon Rank Sum (RS) tests were used where appropriate. **Results:** 14 pregnancies met criteria, had complete data, and were evaluated. Transgenic females (n=10; either hRen or hAng) were more likely than WT females (n=4) to experience spontaneous maternal death (RS p=0.005) and exhibit toxic appearance (RS; p=0.038) but not maternal seizures (RS p=0.076). There were no adverse maternal outcomes in WT pregnancies; Figure 1.



Conclusions: Presence of either the hAng or hRen transgene in female dams led to high rates of deterioration in maternal health during gestation. These data suggest biological activity exhibited by the respective human RAS transgenes interacting with mouse native RAS genes in vivo.

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PHLDA2 Gene Polymorphisms and the Risk of Hypertensive Disorders of Pregnancy and HELLP Syndrome. Aaron J Epstein,[1] Sue A Ingles,[2,3] Deborah A Wing,[1] Melissa L Wilson.[2,3] *[1]Maternal Fetal Medicine, University of California, Irvine, Orange, CA, USA; [2]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA; [3]Department of Preventative Medicine, University of Southern California, Los Angeles, CA, USA.*

Background: PHLDA2 is a functionally imprinted gene that has been implicated as a negative regulator of fetal and placental growth in humans. The aim was to determine whether single nucleotide polymorphisms (SNPs) in the PHLDA2 gene are associated with hypertensive disorders of pregnancy (HDP) in a case-control study of mother-child-dyads or HELLP Syndrome in a case-only study of mother-father-child triads.

Methods: Women with HDP (N=129) and controls (N=167) were recruited from LAC+USC Hospital. Women with HELLP syndrome (N=150) were recruited using an internet based method. DNA was genotyped for four polymorphisms in the PHLDA2 gene using TaqMan assays. These were examined for association with HDP using logistic regression and for HELLP using the Likelihood Ratio Test method for case-parent triads.

Results: Maternal genotype at the rs1129782 locus was associated with risk of HDP, with risk increasing three-fold for each additional copy of the minor (G) allele (OR 3.1 1.7-5.4 P<0.01). The rs1056819 SNP also showed a borderline-significant association with HDP risk (OR 0.5 CI 0.2-1.03 P=0.06). After adjusting for fetal genotypes, maternal effects become more significant for both rs1129782 (OR 4.2 CI 2.0-8.5 P<0.01) and rs1056819 (OR 0.3 CI 0.1-0.8 P=0.02). In the HELLP study, homozygous carriage of the rs1056819 variant allele was associated with decreased risk (OR 0.3 CI 0.06-1.0 P=0.05), consistent with the HDP study. There was evidence of a parent-of-origin effect, with maternally inherited alleles being more common among children of HELLP cases than paternally inherited alleles (p=0.09).

Log-Additive Models for SNPs from Hypertensive Disorders of Pregnancy vs. Controls

Maternal Samples			Child Samples	
SNP	Adjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
RS1056819	0.5 (0.2-1.0)	0.06	0.6 (0.3-1.3)	0.23
RS1129782	3.1 (1.7-5.4)	<0.01	2.3 (1.2-4.4)	<0.01
RS13390	0.8 (0.4-1.9)	0.67	0.8 (0.4-1.9)	0.68
RS2583435	1.0 (0.7-1.6)	0.85	0.9 (0.6-1.4)	0.68

Separate models for mother and child. All genotypes are mutually adjusted for each other.

Conclusion: Mothers carrying the variant allele at rs1056819 in the PHLDA2 gene were less likely to develop HDP and HELLP Syndrome. Variations in the fetal genotypes did not affect their risk.

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Race/Ethnicity Difference in the Associations between Preeclampsia and Acute Renal Failure. Darios Getahun,[1] Michael J Fassett,[2] Deborah A Wing,[3] Steven J Jacobsen.[1] *[1]Department of Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA, USA; [2]Department of Obstetrics & Gynecology, Division of Maternal-Fetal Medicine, Kaiser Permanente West Los Angeles Medical Center, Los Angeles, CA, USA; [3]Department of Obstetrics & Gynecology, University of California Irvine, Orange, CA, USA.*

OBJECTIVE: To evaluate the association between preeclampsia and acute renal failure (ARF) based on maternal race/ethnicity.

METHODS: The 1991-2009 KPSC-Matched Perinatal Service System and Hospital Inpatient record, including birth certificate records for all KPSC births and ICD-9 codes from maternal hospitalizations in all KPSC hospitals, was used to examine the association between preeclampsia with ARF among births at ≥20 weeks of gestation (n= 544,757). The effects of race/ethnicity and gestational age on the association were examined. Odds ratio (OR) and 95% confidence interval (CI) were used to quantify the association after adjustment for confounders.

RESULTS: Rates of preeclampsia among non-Hispanic White (NHW), Black, Hispanic, and Asian/Pacific Islander (A/PI) women were 5.2, 7.7, 5.2, and 4.7, respectively (p<0.01). There were 132 (0.02%) cases of ARF identified during the study period. There were significant differences in race/ethnicity, extremes of parity, and advanced age (p<0.01) between groups. Women with preeclampsia were more likely to be diagnosed with gestational diabetes (p<0.01) and preterm delivery (p<0.01). Term preeclampsia was significant associations with ARF across all racial/ethnic groups [NHW (OR 16.0 95% CI 4.5-57.3), Black (OR 6.4, 95% CI 1.7-23.7), Hispanics (OR 13.0, 95% CI 5.5-30.6), and A/PI (OR 7.0, 95% CI 1.3-37.0)]. Preterm preeclampsia was associated among NHW (OR 9.1, 95% CI 3.0-27.9) and Hispanics (OR 10.7, 95% CI 4.3-26.6) with ARF.

CONCLUSION: The findings of this study suggest that identifying patient at-risk of ARF may be useful for predicting, better monitoring, and avert the dire consequence of preeclampsia.

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Long-Term Risk To Develop Hypertension in Formerly Preeclamptic Women. Chahinda Ghossein,[1] Sander van Kuijk,[2] Julia Spaan,[1] Marc Spaandermand,[1] Tammo Delhaas,[3] Louis LH Peeters.[1] *[1]Obstetrics & Gynecology, Maastricht University Medical Center, Maastricht, Limburg, Netherlands; [2]Epidemiology, Maastricht University Medical Center, Maastricht, Limburg, Netherlands; [3]Biofysical Engineering, Maastricht University Medical Center, Maastricht, Limburg, Netherlands.*

Introduction. Hypertension (HT) develops 4 times more often in formerly preeclamptics than in healthy parous controls. Whether this higher risk applies to all former patients or only to a certain sub-population is unknown. This study in healthy formerly preeclamptic women aims to explore, whether specific abnormalities observed at one-year post partum (pp) can identify those predestined to develop HT in the subsequent period of 13 years.

Methods. At 1 year, and again at 14 years pp, we performed echocardiography and determined a number of metabolic variables in 20 formerly preeclamptics

and 7 parous controls. Post hoc, we divided the former patients into a subgroup who had - (HT-exPE), and a subgroup who had not developed HT (NT-exPE) at 14 years pp. These 3 subgroups were than compared with one another using ANOVA at both time points. In addition, we evaluated, whether the age-dependent change in the metabolic variables differed between the 3 subgroups.
Results. HT-exPE had experienced more often early-onset preeclampsia compared to the NT-exPE (100% Vs 46%) and had developed more often a recurrent hypertensive disorder in pregnancy than NT-exPE (86% Vs 22%). Moreover, HT-exPE had higher systolic and diastolic blood pressures at 1-year pp then NT-exPE, although within the cutoff values for hypertension (130 (95% CI 120; 139) Vs 117 (95% CI 112; 122) mmHg, p<0.05) and (85 (95% CI 76; 92) Vs 70 (95% CI 64; 75) mmHg, p<0.05) respectively.
Although at 1 year pp, HT-exPE had similar left ventricular (LV) geometry as NT-exPE and controls, at 14 years pp HT-exPE had deviated from NT-exPE by signs of LV hypertrophy.
Conclusion. Early-onset preeclampsia, recurrent hypertensive pregnancy and several characteristics of metabolic syndrome are predictive in formerly preeclamptic women with respect to the development of chronic HT. Cardiac aging in former patients who remain normotensive does not differ from that in parous controls, whereas HT-exPE patients develop LV hypertrophy as generally observed in chronic hypertension.

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Characterization of Decoy Receptors D6 and DUFFY in Preeclamptic Placental Tissues. D Giuffrida,[1] A Rolfo,[1] E Piccoli,[1] S Cardaropoli,[1] AM Nuzzo,[1] C Tersigni,[2] N Di Simone,[2] T Todros.[1] *[1]Obstet. and Gynaecol., University of Turin, Turin, Italy; [2]Obstet. and Gynaecol., Catholic University, Rome, Italy.*
Objectives: Preeclampsia (PE) is a pregnancy-related syndrome characterized by exacerbated placental-maternal inflammatory response. Pro-inflammatory chemokines play a pivotal role in inducing inflammation. Their biological effects are inhibited by D6 and DUFFY decoy receptors, deputed to degradation of chemokines and expressed following inflammatory stimuli. Our hypothesis is that aberrations of expression and/or functionality of these receptors could cause or contribute to chemokines accumulation typical of PE. The aims of the present study are to investigate D6 and DUFFY expression and localization in PE placentae and to evaluate maternal serum levels of D6/DUFFY-related chemokines, in order to assess the contribution of these receptors to PE pathogenesis.
Methods: Placental, umbilical cord and placental membranes biopsies were collected from PE (n=10) and control (CTRL, n=10) pregnancies. Maternal serum samples were obtained from the same study population. D6 and DUFFY mRNA expression was assessed by Real Time PCR while their localization was investigated by immunohistochemistry (IHC). Serum samples were processed by Chemokine Array to detect levels of circulating D6/DUFFY-related chemokines.
Results: No differences were found in D6 and DUFFY mRNA expression levels in PE vs CTRL placentae. IHC showed strong D6 and DUFFY signals in both syncytiotrophoblast and membranes of PE and CTRL placentae. No D6 signal was detected in PE and CTRL umbilical cord tissues, while DUFFY signal was stronger in the PE umbilical vein endothelium vs CTRL. Chemokine Array revealed an overall reduction of D6/DUFFY related chemokines in PE vs CTRL maternal serum samples. Specifically, we found significantly decreased levels of Monocyte Chemoattractant Protein 3 (MCP3; p=0.04), chemokine recognized by both D6 and DUFFY, and Macrophage-derived Chemokine (MDC; p=0.029), molecule specifically targeted by D6, in PE vs CTRL serum samples.
Conclusions: Our data of unchanged D6/DUFFY expression in PE placentae, characterized by increased inflammation, are indicative of abnormal decoy receptors activity. Increased DUFFY in PE umbilical vein endothelium suggests an inflammatory response directed towards the fetus. Reduction of MCP3 and MDC molecules in PE placentae implies other pathways in the maintenance of chemokines homeostasis. Further investigation is required.

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Polymorphism of the Epoxide Hydrolase 1 (EPXH1) – Impact on the Severity of Hypertensive Pregnancy Disorders. Tanja Groten,[1] Ekkehard Schleussner,[1] Thomas Lehmann,[2] Robert Zeillinger.[3] *[1]Obstetrics and Gyncecology, University Hospital Jena, Jena, Germany; [2]Institute of Medical Statistics and Computer Science, University Hospital Jena, Jena, Germany; [3]Department of Obstetrics & Gynecology, University of Vienna, Austria.*
OBJECTIVES: With an incidence of 3-7% in all pregnant women preeclampsia is still the leading cause of fetal and maternal mortality. Clinical studies have documented a familiar tendency to develop preeclampsia. Additionally patients with impaired endothelial health are at higher risk. Thus we studied genetic polymorphism of endothelial health related genes in women with and without preeclampsia.
METHODS: 241 African and 279 Caucasian women were recruited in a two hospital-based case-control study for genetic testing of the polymorphisms Epoxide Hydrolase 1 (EPXH1) (codon 113, Tyr/His), Endothelial Nitric Oxide Synthase (codon 298, Glu/Asp), Angiotensinogen (codon 235, Met/Thr) and the Estrogen Receptor 1 polymorphism in intron 1 at position –401 T/C.
RESULTS: From the 241 African women, 95 developed preeclampsia including 19 evolving eclampsia. From the 279 Caucasian women 81 had preeclampsia including 28 developing a HELLP (hemolysis, elevated liver enzymes and low platelets)-syndrome. There was no difference in polymorphism distribution between cases and controls. However we could show a statistical significant association of the EPHX polymorphism encoding Histidin with the severe courses of the disease. 85% His/His, 5% His/Tyr, 0% Tyr/Tyr in African women with eclampsia vs 67% His/His, 25% His/Tyr und 5% Tyr/Tyr in those with preeclampsia alone (p=0,026) and 61% His/His, 36% His/Tyr, 3,6% Tyr/Tyr in Caukasian women developing a HELLP-syndrome vs 44% His/His, 40% His/Tyr und 17% Tyr/Tyr in preeclampsia alone (p=0,045).
CONCLUSIONS: EPXH plays an important role in both activation and detoxification of exogenous chemicals. The 113 Tyr/His exchange leads to decreased enzymatic activity interfering the capability of detoxification especially in endothelial cells which is believed to be part of the pathomechanism of preeclampsia. Our data implicate the genetic background to impact on the course of the disease and alter the capability of the maternal organism to deal with the pregnancy derived agents causing preeclampsia.

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Decreased Numbers of Hofbauer Cells (HBCs) in Placentas from Pregnancies with Severe Preeclampsia (PE): Potential Pro-Inflammatory Consequences. Seth Guller,[1] Zhonghua Tang,[1] Irina A Buhimschi,[1] Catalain S Buhimschi,[1] Tracy Niven-Fairchild,[2] S Joseph Huang,[1] Serkalem Tadesse,[2] Errol R Norwitz.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, New Haven, CT; [2]Obstetrics & Gynecology, Tufts Medical Center, Boston, MA.*
OBJECTIVES: Alterations in placental immune function are suggested to play a role in activating pro-inflammatory pathways in preeclampsia (PE). Like other tissue macrophages, HBCs (fetal macrophages) mediate innate immune responses within the placenta, and are classified as M1 (pro-inflammatory) or M2 (anti-inflammatory/pro-angiogenic). This study investigates for the first time whether: (i) absolute levels of HBCs and ii) HBC phenotypes are altered in placentas obtained from pregnancies with severe preterm PE.
METHODS: Placentas were collected from pregnancies with severe preterm PE (n=10) and gestational age-matched spontaneous preterm birth (sPTB, n=11) (gestational age 30±1 weeks for both; mean±SEM). Immunohistochemistry (IHC) and quantitative real-time PCR (qRTPCR) were used to assess placental levels of M1 (CD11b/ITGAM, CD40), M2 (folate receptor-β (FR-β), CD163) and pan (CD68) macrophage markers. To determine macrophage phenotypes, cultures of HBCs with ≥ 98% purity were isolated from term placentas (n=3) using Percoll gradients and negative immunoselection techniques, and analyzed by flow cytometry.
RESULTS: Quantitation of IHC results revealed significant 28%, 34%, and 58% decreases in the numbers of FR-β^+, CD163$^+$, and CD68$^+$ HBCs, respectively in the placental stroma of the PE group compared to the sPTB group (P<0.001 for all). qRTPCR confirmed the expression of FR-β, CD163, and CD68 mRNA was reduced by 73% (P<0.001), 79% (P<0.05) and 59% (P=0.06), respectively in the PE group compared to the sPTB group. Flow cytometry revealed that HBC cultures expressed high levels of FR-β and CD163, but did not express CD11b or CD40 macrophage markers, demonstrating that HBCs are M2 macrophages.
CONCLUSIONS: These results indicate that severe preterm PE is associated with a decrease in HBCs which express an M2 (anti-inflammatory) phenotype.

Decreased numbers of HBCs may promote pro-inflammatory processes and the release of placental factors into the maternal circulation which aberrantly activate vascular endothelia leading to PE.

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Hypertension and Placental Dysregulation in Vitamin D-Deficient Pregnant Mice. Nancy Q Liu,[1] Yi Ouyang,[2] Venu Lagishetty,[1] Bruce W Hollis,[3] Ozlem Equils,[4] Martin Hewison.[1] *[1]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA; [2]Department of Pathology, Veterans Affairs Medical Center, Long Beach, CA, USA; [3]Medical Division, Pfizer Inc., New York, NY, USA; [4]Division of Neonatology, Medical University of South Carolina, Charleston, SC, USA.*

Association studies suggest that preeclampsia (PE) is linked to vitamin D-deficiency. To determine whether vitamin D status contributes to the pathophysiology of PE, we carried out studies using pregnant BL6 mice raised on vitamin D-sufficient or -deficient diets. Female mice were fed one of these diets from weaning (wk 4) until wk 8. They were then mated with vitamin D-sufficient males, and the resulting pregnant mice either: 1) allowed to deliver pups and monitored for blood pressure (BP) and weight of offspring; 2) euthanized at day 14 or 18 of gestation for analysis of serum, placental/kidney tissues, and fetus. At day 14 of gestation serum levels of precursor 25-hydroxyvitamin D (23.0 ± 2.3 *vs* 0.5 ± 0.03 ng/ml) and active 1,25-dihydroxyvitamin D (105.8 ± 7.0 *vs* 42.4 ± 9.5 pg/ml) were higher in vitamin D-sufficient *vs* –deficient mice. Analysis of BP by tail-cuff showed no significant difference between vitamin D-sufficient *vs* deficient mice at baseline (immediately prior to mating). However, at day 14 of gestation BP was significantly elevated in vitamin D-deficient mice (124.9/105.8 ± 2.5/1.9 mmHg) relative to vitamin D-sufficient mice (105.3/76.3 ± 3.6/5.0 mmHg). This continued throughout pregnancy and wk 1 postpartum, but returned to baseline by wk 2 postpartum. Analysis of kidney tissue showed elevation of mRNA for renin and the angiotensin II receptor (3- and 4-fold respectively) in vitamin D-deficient *vs* –sufficient mice at day 14 of gestation. Day 14 placentas from vitamin-deficient mice showed pronounced histological changes and DNA array analyses identified differentially expressed genes, including placental growth factor, associated with this. Analysis of fetal development showed that day 14 and 18 fetuses from vitamin D-deficient mice were larger (length and weight) than those from -sufficient mothers. However, by wk 2 postpartum pups from vitamin D-deficient mothers weighed significantly less than those from –sufficient mothers. These data provide further evidence of a pivotal role for vitamin D in pregnancy. In particular, we propose that low vitamin D status may predispose to PE and dysregulation of fetal development.

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The RNA Levels of Keys Enzimes for the Methionine-Homocysteine Metabolism Are Elevated in Placenta of Preeclamptic (PE) Patients. Francisco J Valenzuela, Alejandra Perez-Sepulveda, Maria J Torres, Jose Galaz, Alejandra Guzman, Francisca Mena, Horacio Figueroa-Diesel, Sebastian Illanes. *Laboratorio Biología de la Reproducción. Facultad de Medicina, Universidad de los Andes, Santiago, RM, Chile.*

Methionine-homocysteine metabolism (MHM) is critical in the availability of methionine (Met) that is essential for placental/fetal development. Defects in methylation pathway reduce the availability of Met, needed for the production of S-adenosylmethionine (SAM), donor of CH3-groups and critical for conversion of 2-OH-estradiol to 2-methoxyoestradiol (2-ME) by catechol O-methyltransferase (COMT). Low levels of (2-ME) have been reported in PE women. Hypothesis: Alterations in MHM via low levels of mRNA expression of methionine synthase (MTR), methionine synthase reductase (MTRR) and methylenetetrahydrofolate (MTHFR) are associated with low levels of 2-ME in PE. Methods: Pregnant women (control=14 and PE=7) were recruited from Hospital Parroquial de San Bernardo after ethical consent signature. Total RNA was obtained with RNA purification kit (Master Pure, Epicentre). Real Time PCR was performed for MTHFR, MTRR, MTR and 18S-rRNA using specific probe, the products were interpolated using a purified standard curve and expressed as fg/ng RNA. The mRNA levels were analyzed using Mann Whitney test. Results: The mRNA levels of MTHFR and MTR were higher in patients with PE compared with control (P<0.05) and there was a trend to higher levels of MTRR in PE than control that no reached significance.

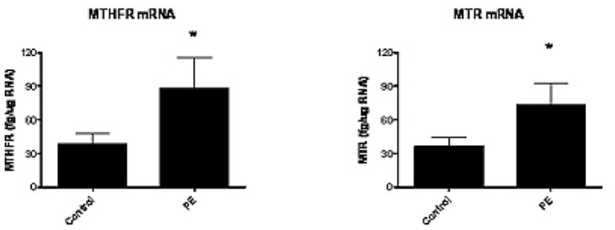

*Figure 1: Expression of MTHFR and MTR mRNA in placenta of patient with control (n=14) or PE (n=7). *.P<0.05 Mann Whitney test.*

Conclusion: The elevated levels of MTHFR and MTR are in contrast with previous reports showing low levels of 2-ME in PE, and suggest other possible enzymatic pathway to explain the low level of 2-ME. High levels of MTHFR and MTR in PE can be a physiological response to low levels to 2-ME and could explain the hyperhomocysteinemia observed in PE.

Supported by FONDECYT 1110883.

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Quantitative Study of Plasma Profiles of Estrogens and Estrogen Metabolites in Normotensive Pregnancy, Mild Preeclampsia and Severe Preeclampsia by Liquid Chromatography Mass Spectrometry. Sheikh O Jobe, Chanel T Tyler, Ronald R Magness. *Obstetrics and Gynecology, University of Wisconsin-Madison.*

Introduction: 2-methoxyestradiol (2-ME2), a metabolite of estradiol-17β (E2) synthesized from catecholestrogens by catechol-O-methyltransferase may play a critical role in the pathophysiology of preeclampsia. However, plasma levels of specific estrogens and/or estrogen metabolites remain undetermined and patterns of estrogen metabolism during normal (NP) and preeclamptic (PE) pregnancies are unclear. Objective: To compare plasma concentrations of total estrone (E1), E2, estriol (E3), 2-hydroxyestrone (2-OHE1) and 4-hydroxyestrone (4-OHE1), 16-hydroxyestrone (16-OHE1), 2-hydroxyestradiol (2-OHE2), 2-methoxyestrone (2-ME1), 3-methoxyestrone (3-ME1), 4-methoxyestrone (4-ME1), 2-ME2, 4-methoxyestradiol (4-ME2), 16-Keto-estradiol (16-Keto-E2), 16-epi-estriol (16-E3) and 17-epi-estriol (17-E3) in NP women to those of women with mild (mPE) and severe (sPE). Methods: Plasma samples were obtained from NP (n = 4), mPE (n = 4) and sPE (n = 4). Quantitative levels of estrogens and estrogen metabolites were measured by liquid chromatography mass spectrometry. Results: Compared to plasma levels of E1 and E2 in NP (50558±102, 8891±89 pg/ml respectively), levels in sPE (2631±102, 2039±89 pg/ml respectively) but not in mPE were lower (P< 0.001). Compared to levels of 2-OHE1 and 2-OHE2 in NP (490±53, 487±39 pg/ml respectively), levels were lower (P<0.001) in mPE (201±53, 221±39 pg/ml respectively) and sPE (176±53, 206±39 pg/ml respectively). 4-OHE1 and 16-OHE1 were unchanged. Compared to levels of 2-ME1, 4-ME1, 2-ME2 and 4-ME2 in NP (823±52, 698±78, 1967±47, 1151±90 pg/ml respectively), levels were lower (P<0.001) in sPE (508±52, 526±79, 957±47, 434±90 pg/ml respectively) but not in mPE (889±51.9, 734±78.2, 1988±46.9, 1434±89.4 pg/ml respectively). Interestingly, compared to plasma levels of 16-Keto-E2 in NP (883± 53pg/ml) and mPE (788± 53pg/ml), levels were higher in sPE (1939 ± 53 pg/ml). E3, 16-E3 and 17-E3 were unchanged. Conclusions: Since estrogens and estrogen metabolites play key roles in cardiovascular adaptations during normal pregnancy, these data suggest that aberrant synthesis and metabolism of estrogens may be a mechanism responsible for the dysfunctional cardiovascular function in pregnancies complicated with preeclampsia. NIH HL49210, HD38843, HL87144, T32-HD041921

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Antithrombotic Therapy and Placental Implantation-Related Adverse Pregnancy Outcomes. Joel D Kamda,[1] Alessandro Ghidini,[2] Helain J Landy,[1] Jim C Huang,[3] Sarah H Poggi.[1,2] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, INOVA Alexandria Hospital, Alexandria, VA, USA; [3]Statistics, Medstar Research Institute, Hyattsville, MD, USA.*

OBJECTIVE: To determine if antithrombotic treatment is associated with reduced risk of recurrence of placenta-related adverse pregnancy outcomes (APO) irrespective of thrombophilia status.

STUDY DESIGN: This is a retrospective 5 year cohort study of pregnant women (N=128) with history of APOs including severe preeclampsia (n=28), placental abruption (n=4), fetal loss >20 wks (n=47), recurrent spontaneous abortions (n=25) and intrauterine fetal growth restriction (n=24). Workup for thrombophilias and administration of low molecular weight heparin (LMWH) or

low dose aspirin (ASA) prophylaxis were left at the discretion of the managing physician. Excluded were cases requiring LMWH prophylaxis for medical indications. Patients experiencing recurrent APO were compared to those who did not using Chi-square, Student t-test and logistic regression analysis.
RESULTS: The overall recurrence of APO was 21% (27/128). Univariate anlaysis revealed significant differences in maternal age (31.9 +/- 6.3 vs 34.6 +/- 5.1, P=0.02), LMWH (11 % vs 37%, OR=0.22, 95% CI 0.05-0.83), and ASA (7% vs 31%, OR=0.18, 95% CI 0.03-0.86) between women with vs without recurrent APO. Logistic regression analysis was unable to assess the independent effect of LMWH and ASA on APO due to co-linearity between the two variables
CONCLUSION: In a cohort of patients with rigorously defined history of placental implantation related APO, antithrombotic treatment was associated with a significant reduction in recurrence of APO in a subsequent pregnancy. The independent effect of LMWH vs ASA requires a series of patients exposed to either therapy only.

F-323

Haemostatic and Angiogenic Dysregulation in Preeclampsia. Lynne A Kelly,[1] Shanthi Muttukrishna,[1] Lucy A Norris,[2] John R Higgins.[1] [1]*Anu Research Center, Obstetrics and Gynaecology, University College Cork, Ireland;* [2]*Obstetrics and Gynaecology, Trinity College Dublin, Ireland.*
Pre-eclampsia (PE), affecting between 3-5% of pregnant women, is a major contributor to perinatal and maternal morbidity and mortality worldwide. In normal pregnancy, an activation of haemostasis occurs to prevent blood loss during childbirth and angiogenesis is vital for placentation. Previous work has suggested that the balance between haemostasis and angiogenesis may be critical for pregnancy outcome. A small number of studies have reported conflicting results and further investigations are necessary to define the inter-relationship between regulation of haemostasis and angiogenesis at a molecular level in the placenta.
The objective of this ongoing study is to investigate placental expression of the haemostatic and angiogenic genes and proteins and to evaluate their relationship in PE.
Placental tissue was collected at term (≥37 weeks of gestation) from PE patients (n=9) and gestation matched normal control pregnancies (n=9) and snap frozen within 30 minutes of delivery of the placenta. Biopsies were stored at -80^{o}C until RNA extraction. cDNA was synthesised and Real Time PCR was carried out for relative quantification of specific genes. Total protein was extracted and proteins were measured using ELISA. Statistical analysis was performed using non parametric Mann-Whitney U test. Correlation between parameters was assessed using the Spearman rank test. Values of $P \leq 0.05$ were considered significant.
The preliminary results of the study show that tissue factor mRNA levels are reduced ($P<0.001$) while TFPI-2 is increased ($P<0.05$) in PE compared to normal pregnancy. PAI-1 and PAI-2 are also increased in PE versus normal pregnancy ($P<0.01$). TFPI-1, VEGF A, PlGF and sFLT-1 were not significantly altered in term PE. Protein results show a similar trend to that of RNA for all molecules. Correlation analysis has shown a significant relationship between tissue factor and VEGF-A ($\rho=0.75$, $P<0.02$) and also TFPI-1 and PlGF ($\rho=0.67$, $P<0.05$) in PE placenta.
This study shows the disturbances in the regulation of some haemostatic genes in term PE placenta compared to controls. The interplay between haemostasis and the angiogenic pathway is crucial in pregnancy and the possible dysregulation shown here may contribute to the development of preeclampsia. However, larger sample numbers are required to confirm these observations.

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A High Throughput and Accurate Early Pregnancy-Screening Test for Preeclampsia. Louise C Kenny,[1] David I Broadhurst,[2] Jenny E Myers,[3] Robyn A North,[4] Philip N Baker.[2] [1]*The Anu Research Centre, Department of Obstetrics and Gynaecology, University College Cork, Ireland;* [2]*Department of Medicine, University of Alberta, Canada;* [3]*Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom;* [4]*Division of Women's Health, King's College London, United Kingdom.*
Introduction
We have previously presented to the Society, and subsequently published [1] a case-control study, which putatively identified a unique set of 14 plasma metabolomic biomarkers with the potential to accurately predict preeclampsia in early pregnancy. Here we present an algorithm generated using a reduced number of these metabolites, combined with limited clinical data, that we believe offers a robust, sensitive and specific screening methodology for preeclampsia.
Methods
The exact chemical identity and absolute quantification of the original set of metabolites was obtained using Liquid Chromatography Triple Quadrupole Mass Spectroscopy (LC-QQQ-MS). Each metabolite was quantified in plasma samples taken at 15 weeks' gestation from a cohort study of 2077 low risk nulliparous women enrolled in the SCOPE study (www.scopestudy.net) in the UK and Ireland. An algorithm to predict the subsequent development of preeclampsia and which combined absolute levels of a reduced set of metabolites and limited clinical data (age, body mass index and blood pressure) was developed using multivariate machine-learning and evolutionary optimization methods.
Results
There were 80 women who developed preeclampsia in the prospective cohort of 2077 UK and Irish women (overall incidence of 3.9%). Each sample took less than 10 minutes to process using LC-QQQ-MS; detection limits ranged from 1-600 ng/mL, (median 36 ng/mL). The algorithm predicted preeclampsia at 15 weeks' gestation with a test sensitivity of 92% and a specificity of 95%. The exact formula of the algorithm will be presented in full.
Discussion
Here we present a simple algorithm based on metabolomic biomarkers, which offers the promise of a high throughput early pregnancy-screening test for preeclampsia with unprecedented sensitivity and specificity. Moreover, LC-QQQ-MS offers a single platform with a one-stage sample preparation and is already in use in newborn screening programs across Europe and US. This will streamline regulatory approval for adaptation for use in preeclampsia and we thus plan to launch a phase IIa clinical study in 2012.
1. Kenny et al. Hypertension. 2010 Oct;56(4):741-9.

F-325

Maternal Hemodynamics at 11-13 Weeks of Gestation and the Risk of Preeclampsia. Asma Khalil,[1] Ranjit Akolekar,[2] Argyro Syngelaki,[1,2] Mohamed ElKhaouli,[1,2] Kypros Nicolaides.[1,2] [1]*Fetal Medicine, Institute for Women's Health, University College London Hospitals, London, United Kingdom;* [2]*Fetal Medicine, King's College Hospital, London, United Kingdom.*
Objective: Women who develop preeclampsia (PE) are at increased risk of cardiovascular disease and stroke in the subsequent decades. In individuals with cardiovascular disorders there is increased central aortic systolic blood pressure and arterial stiffness, assessed by pulse wave velocity and augmentation index. The aim of this screening study was to examine the potential value of aortic systolic blood pressure, pulse wave velocity and augmentation index at 11-13 weeks' gestation in identifying women who subsequently develop PE.
Methods: This was a prospective screening study for PE at 11+0-13+6 weeks' gestation. The inclusion criteria were women with a singleton pregnancy and a live fetus identified at the 11+0-13+6 weeks scan. We excluded pregnancies with major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks. Pulse wave velocity, augmentation index and aortic systolic blood pressure were measured. We examined the performance of these parameters in screening for PE. The distributions of AIx, PWV and SBPAo were made Gaussian after logarithmic transformation. Multiple regression analysis in the unaffected group was used to examine which of the maternal characteristics provided a significant contribution in the prediction of AIx-75, PWV and SBPAo. Each value in the PE, GH and unaffected groups was expressed as a multiple of the unaffected median (MoM) after adjustment for those characteristics.
Results: In the PE group (n=146), compared to unaffected controls (n=4,436), there was an increase in PWV (1.12 vs 1.00 MoM, $p<0.0001$), AIx-75 (1.06 vs 1.00 MoM, $p<0.0001$) and SBPAo (1.10 vs. 1.00 MoM, $p<0.0001$). Logistic regression analysis demonstrated that in the prediction of PE there were significant contributions from log10AIx-75 MoM (OR 4.8E-03, 95% CI 1.6E-04–0.14 p=0.002), log10PWV MoM (OR 279.3, 95% CI 27.1–2.9E03; $p<0.0001$) and log10SBPAo (OR 2.7 E07, 95% CI 4.7E06 – 15.1E09, $p<0.0001$). In screening for PE by a combination of maternal variables and log10 AIx-75 MoM, log10PWV MoM and log10 SBPAo, the estimated detection rate was 61.6% (95% CI 51.8-70.5) at a false-positive rate of 10%.
Conclusion: A high proportion of women who develop PE have increased aortic systolic blood pressure and arterial stiffness apparent from the first-trimester.

Friday

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Cardiovascular Disease Risk Factors in Women with a History of Early Onset Versus Late Onset Preeclampsia and Pregnancy Induced Hypertension. Anath Y Breimer,[1] Maria PH Koster,[1] Wietske Hermes,[2] Christianne JM de Groot,[3] Ben W Mol,[4] Bas B van Rijn,[1] Arie Franx.[1] *[1]Obstetrics, University Medical Centre Utrecht, Netherlands; [2]Obstetrics, Leiden University Medical Centre, Netherlands; [3]Obstetrics, Free University Medical Centre Amsterdam; [4]Obstetrics, Academic Medical Centre Amsterdam, Netherlands.*

Objective

Previous studies showed an increased risk of cardiovascular disease (CVD) in early-onset preeclampsia (EOPE) as compared to late-onset preeclampsia (LOPE). Whether this difference is reflected in the presence of CVD risk factors postpartum is unclear. Our objective was to compare CVD risk factors between women with a history of EOPE, LOPE and pregnancy induced hypertension (PIH).

Methods

We studied women who experienced EOPE (n=81), LOPE (n=75) and PIH (n=218). CVD risk factors measured were blood pressure (BP), lipid profile, fasting blood glucose levels, BMI and hsCRP. We used Kruskal-Wallis tests for statistical comparison.

Results

Age, BMI, use of hypertensive medication, systolic and diastolic BP, fasting glucose levels, HDL cholesterol and hsCRP differed significantly across groups. Effects of the difference in follow-up time will be examined in subsequent analyses.

Baseline characteristics

	EOPE (n=81)	LOPE (n=75)	PIH (n=218)	p
Age, yrs	31 (27-35)	34 (31-38)	33 (30-37)	0.001
BMI, kg/m2	25 (22-29)	25 (22-29)	27 (24-31)	<0.001
Hypertensive medication	18 (22.2)	6 (8.0)	20 (9.2)	<0.001
Smoking	10 (12.3)	15 (20.0)	42 (19.3)	0.262
Diabetes	0	1 (1.3)	0	-
Follow-up, days	134 (111-181)	888 (803-977)	879 (797-1011)	<0.001
Blood pressure, mmHg				
Systolic	125 (115-130)	120 (110-130)	125 (118-130)	0.018
Diastolic	80 (70-85)	80 (70-85)	84 (80-90)	<0.001
Fasting blood glucose, mmol/l	5.3 (5.0-5.5)	4.7 (4.5-5.0)	4.8 (4.5-5.1)	<0.001
hsCRP, mg/l	1.85 (0.60-4.95)	1.43 (0.69-4.47)	2.57 (1.08-5.74)	0.019
Lipid profile, mmol/l				
Total cholesterol	4.70 (4.25-5.10)	4.70 (4.10-5.43)	4.70 (4.18-5.30)	0.970
LDL	3.13 (2.73-3.65)	3.11 (2.66-3.88)	3.03 (2.59-3.67)	0.707
HDL	1.24 (1.09-1.51)	1.30 (1.10-1.50)	1.40 (1.20-1.60)	0.001
TG	0.80 (0.60-1.30)	0.87 (0.64-1.39)	0.91 (0.66-1.21)	0.698
Chol/HDL ratio	3.59 (2.97-4.56)	3.56 (3.04-4.21)	3.36 (2.85-4.09)	0.051

Data are given as median (IQR) or n (%)

Conclusion

Different CVD risk factor patterns were observed after EOPE, LOPE and PIH. Women who experienced EOPE have increased blood glucose levels and more often use hypertensive medication as compared to women who had LOPE. Women who had PIH had highest BMI and diastolic BP. Prevention of CVD may require different approaches after EOPE, LOPE and PIH.

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Attenuation of VEGFR-2 Expression by Soluble VEGFR-1 (sFlt-1) and Hypoxia in the Human Placenta. Dennis K Lee,[1] Isabella Caniggia,[2] Ori Nevo.[1] *[1]OB/GYN, Sunnybrook HSC, University of Toronto; [2]OB/GYN, Mount Sinai Hospital, SLRI, Toronto, Canada.*

Objective: Vascular endothelial growth factor receptor 2 (VEGFR-2) is the primary receptor for VEGF, is crucial for normal endothelial function and is reduced in placenta from patients with preeclampsia (PE). sFlt-1, which binds and inhibits VEGF, is increased in PE and is positively regulated by low oxygen. Our **objective** was to examine the effect of sFlt-1 on VEGFR-2 expression and signaling in the human placenta. **Methods:** Placental samples were collected from early onset PE (n=27) and control (n=16) pregnancies. First trimester villous explants were cultured at 3% or 20% O_2 in the presence of sFlt-1 (n=4). VEGFR-2 protein and mRNA expression were measured by western blot and quantitative real-time PCR analyses respectively. Protein interaction was examined by co-immunoprecipitation (Co-IP) and co-localization by immunofluorescence (IF) for VEGFR-2 and sFlt-1. **Results:** VEGFR-2 transcript and protein levels were significantly decreased in PE placentae compared to controls (1.82 and 1.85 fold, respectively). An inverse correlation was observed for VEGFR-2 and sFlt-1 levels in both singleton and twin placentae from patients with PE. IF analyses revealed co-localization of VEGFR-2 and sFlt-1 in placental vasculature and Co-IP analyses confirmed VEGFR-2 and sFlt-1 interaction only in PE placentae compared to age-matched controls. VEGFR-2 transcript and protein levels from explants cultured in 3% O_2 (known to be associated with increased sFlt-1 expression) were significantly decreased compared to those incubated at 20% O_2 (5.9 and 12.47 fold, respectively). Also, VEGFR-2 expression levels were decreased in early first trimester placentae (low oxygen environment) compared to late first trimester. We next explored whether sFlt-1 directly affects VEGFR-2 expression. Treatment of first trimester placental explants with sFlt-1 resulted in significantly decreased levels of VEGFR-2 (2.03 fold) and downstream signaling proteins phospho-ERK (1.60 fold) and phospho-Akt (1.64 fold). **Conclusions:** Our findings show a novel hypoxia-induced down-regulation of VEGFR-2 in the human placenta. sFlt-1, which is known to be increased in hypoxic conditions, directly attenuates VEGFR-2 expression and signaling. Direct interaction between sFlt-1 and VEGFR-2 may represent an important mechanism in VEGFR-2 regulation, especially in preeclampsia that is associated with a high level of sFlt-1.

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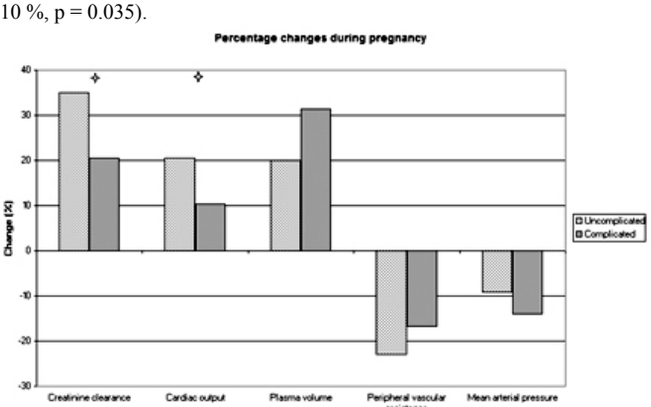
Cardiovascular and Renal Adaptation to Pregnancy in Recurrent Hypertensive Gestation. Veronica A Lopes van Balen, Julia J Spaan, Marc EA Spaanderman, Louis LH Peeters. *Obstetrics & Gynecology, Maastricht University Medical Center, Maastricht, Limburg, Netherlands.*

Introduction: It is generally believed that shallow trophoblast invasion and defective spiral artery remodeling precede the development of hypertensive pregnancy disorders. Whether these abnormalities at the fetal-maternal interface are paralleled by altered systemic maternal cardiovascular changes, is unknown. In this study we compared the maternal cardiovascular response to the next pregnancy between formerly preeclamptic women who did and did not develop a recurrent hypertensive complication in their next pregnancy.

Methods: In this observational cohort study, we enrolled 61 normotensive women whose previous pregnancy had been complicated by a hypertensive disorder. We divided these women into a subgroup with a normal next pregnancy (n=33) and a subgroup who developed a recurrent hypertensive disorder in the next pregnancy (n=28). We measured cardiac output (doppler/ultrasound), blood pressure and heart rate (automated oscillometric device), plasma volume (Dextran-70 dilution), creatinine clearance and microalbuminuria, preconceptionally and again at 18±2 weeks in their next pregnancy.

Result: Groups were comparable with respect to age and BMI. Both subgroups responded to pregnancy with an increase in cardiac output, plasma volume, heart rate and glomerular filtration rate, and a decrease in blood pressure and total peripheral vascular resistance.Women who developed a recurrent hypertensive disorder in their next pregnancy differed from their counterparts with an uneventful next pregnancy by smaller pregnancy-induced increases in creatinine clearance (31% vs. 19%, p =0.035) and cardiac output (20% vs. 10 %, p = 0.035).



Conclusion: The cardiovascular and renal adaptation to pregnancy in women developing a recurrent hypertensive disorder in their next pregnancy differs from that in their counterparts with an uneventful next pregnancy by slightly smaller increases in creatinine clearance and cardiac output.

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Vitamin D Supplementation Improves Pregnancy Outcomes in a Rat Model of Late-Stage Pre-Eclampsia. Eugenia Mata-Greenwood, John Stewart, Ravi Goyal, Lawrence D Longo. *Center for Perinatal Biology, Loma Linda University, Loma Linda, CA.*

We have recently discovered that Brown Norway (BN) rats present fetal resorption linked with maternal vitamin D (VD_3) deficiency and Renin-Angiotensin system activation. Because similar findings have been linked to

preeclampsia, we hypothesized that BN rats would present preeclampsia. We also hypothesized that VD_3 deficiency could originate from maternal renal dysregulation of VD_3 metabolizing enzymes. To prove our hypothesis, we monitored maternal blood pressure, proteinuria, VD_3 activation and maternal renal gene expression in BN rats at various stages, including pre-pregnancy, gestational days 8, 13, 17, 21, and postpartum days 7, 14 and 21. Mean arterial blood pressure, measured by tail-cuff plethysmography was normal (93 ± 10 mmHg) at pre-pregnancy and early-pregnancy stages, but was significantly elevated at day 19 and 21 of pregnancy (117 ± 13 mmHg, $p<0.01$), and correlated with proteinuria. Plasma levels of VD_3 metabolites were then analyzed by ELISA. The maternal plasma levels of the active metabolite of VD_3 (aka calcitriol) showed a 6-fold decrease of pre-pregnancy levels at 21 days gestation, but gradually increased post-partum (30 ± 11 pM at 21 days of gestation vs. 202 ± 59 pM at postpartum day 21, $p<0.001$)). In contrast, the precursor metabolite 25-hydroxyvitamin D_3 remained unchanged throughout gestation and lactation. We studied the gene expression of VD_3 metabolizing enzymes in maternal kidney by real-time PCR. The mRNA expression of the VD_3 activating enzyme CYP27B1 declined with gestational age and returned to basal levels at 7 days postpartum. In contrast, the VD_3 inactivating enzyme CYP24A1 was upregulated at 17 days of gestation. We then chronically administered the active VD_3 metabolite (calcitriol, ~200 pM/day) via osmotic minipump, starting at day 8 of pregnancy and continuing throughout lactation. Exogenous supplementation with calcitriol increased the maternal pregnancy weight gain (80 ± 5.5 vs 49.3 ± 9.6, g, $p<0.05$ vs. solvent only) and significantly increased the litter size (7.3 ± 1.5 vs 3.0 ± 1.7 pups/litter, $p<0.05$ vs. solvent only). We conclude that BN rats are a novel model of late-stage preeclampsia linked to VD_3 deficiency. VD_3 deficiency could be due to renal dysregulation of CYP24A1 and CYP27B1 expression. Finally, VD_3 supplementation improved pregnancy outcomes, therefore future studies of the VD_3 system in this new animal model are warranted.

F-330

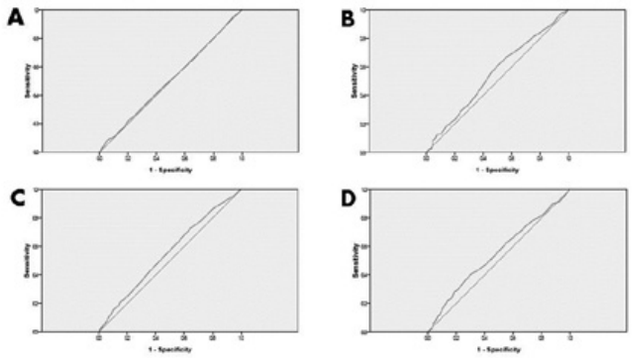
Can Preeclampsia Be Predicted Based on AST and ALT Levels during the First 20 Weeks of Pregnancy? Elad Mei-Dan,[1] Arnon Wiznitzer,[2] Ruslan Sergienko,[2] Mordechai Hallak,[1] Eyal Sheiner.[2] *[1]Obstetrics & Gynecology, Hillel Yaffe Medical Center, Hadera, Israel; [2]Obstetrics & Gynecology, Soroka University Medical Center, Be'er-Sheva, Israel.*

Objective: To examine whether plasma activity levels of the biochemical parameters AST and ALT during the first 20 weeks of pregnancy can predict preeclampsia in the second half of pregnancy.

Methods: The study population included registered births (n=15,010) during 2001-2010 in a tertiary medical center. Receiver operating characteristic curve analysis was used to describe the relationship between the sensitivity (true positive) and the false-positive rate for different values of AST and ALT during the first 20 weeks gestation in the prediction of preeclampsia.

Results: While no significant association was noted between AST and mild preeclampsia (Fig. A; area under curve (AUC) =0.506; SE=0.013, 95% CI 0.14-0.53, P=0.669), elevated levels of AST were significantly associated with severe preeclampsia (Fig. B; AUC =0.506; SE=0.023, 95% CI 0.50-0.60, P=0.027). However, AST level of 50IU/L had a sensitivity of only 2.0%, although a specificity of 98% in the prediction of severe preeclampsia. Elevated ALT levels were significantly associated with both, mild (Fig. C; AUC =0.552; SE=0.023, 95% CI 0.53-0.58, P<0.001) and severe preeclampsia (Fig. D; AUC =0.551; SE=0.013, 95% CI 0.50-0.60, P=0.032). Yet, ALT level of 50IU/L had a sensitivity of only 3.3% and a specificity of 97% in the prediction of severe preeclampsia.

Conclusions. Higher AST and ALT levels during the first 20 weeks of pregnancy are associated with higher risk for the development of severe preeclampsia in the second half of the pregnancy. Nevertheless, there is no clinical cut-off value that can be practically used for the prediction of preeclampsia.



F-331

Levels of the Soluble Lectin-Like Oxidized LDL Receptor (sLOX) in Women with Preeclampsia. Jude S Morton,[1] Tatsuya Sawamura,[2] Ali Abdalvand,[1] Sandra T Davidge.[1] *[1]Obstetrics and Gynaecology, University of Alberta, Edmonton, AB, Canada; [2]Vascular Physiology, National Cerebral and Cardiovascular Centre Research Institute, Suita, Osaka, Japan.*

Introduction

Preeclampsia (PE) is common, complex, disorder of pregnancy, resulting in increased maternal morbidity/mortality and induced preterm delivery. In our laboratory, we made the recent, exciting discovery that oxidized LDL and its receptor (LOX-1) are both increased in the systemic vasculature of women with PE (Hypertension 2009;53:270-7). LOX-1 and its soluble receptor have been shown to be increased in cardiovascular diseases such as atherosclerosis, stress, diabetes and hypertension. We hypothesized that levels of the soluble LOX-1 (sLOX) receptor would be elevated in women with PE compared to normal pregnant women and, if so, may be useful as a biomarker for women with PE.

Methods

All subjects provided informed consent before inclusion in the study. Blood was collected by routine forearm venipuncture at admission (before delivery) from healthy pregnant and women diagnosed with PE. PE (n=12) was characterized by the de novo onset of hypertension and proteinuria after the 20th week of gestation. Hypertension was defined as blood pressure >140/90mmHg on two occasions, 6 hours apart and proteinuria of >+2 on a dip stick. Normal pregnant subjects (n=12) were normotensive throughout pregnancy. Serum and plasma were separated and levels of sLOX and LOX-1 ligand containing ApoB (LAB, thought to best reflect atherogenicity of LDL) were detected by ELISA using antihuman LOX-1 and anti-ApoB antibodies, respectively.

Results

Levels of sLOX-1 were not different between control and PE pregnancies in plasma (499±115pg/ml control, 445 ± 111pg/ml PE) or serum (1434±302 pg/ml control, 1114±201pg/ml PE). Levels of LAB were also not different between control and PE pregnancies in plasma (571±40ng/ml control, 610±44ng/ml PE) or serum (404±21ng/ml control, 448±32ng/ml PE).

Discussion

Contrary to our hypothesis, levels of sLOX or LAB were not altered by PE. Although LOX-1 had been shown to be increased in the vasculature of women with PE, this did not lead to increased levels of the circulating soluble form or receptor ligands. We speculate that, in contrast to other cardiovascular diseases, the relatively short duration of the vascular pathogenesis for women with PE does not result in circulating sLOX-1. Whether there are long term affects of LOX-1 or its soluble receptor in women who had PE remains to be determined.

F-332

Epigenetic Alterations and *MMP1* and *MMP8* Gene Expression in Preeclampsia. Ahmad A Mousa, Sonya L Washington, Jerome F Strauss III, Scott W Walsh. *OB-GYN, Physiology & Biophysics, Virginia Commonwealth University Medical Center, Richmond, VA, USA.*

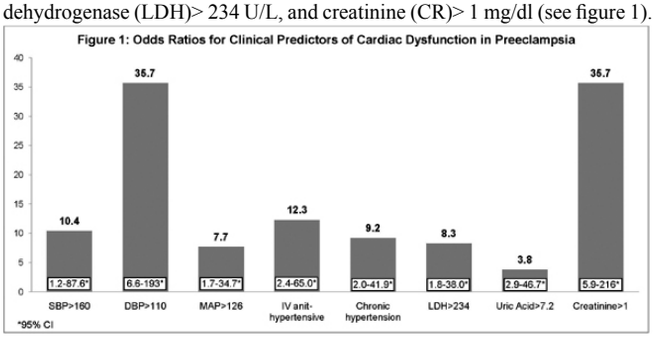
Introduction: Matrix metalloproteinase-1 (MMP-1) and MMP-8 are matrix degrading enzymes that are increased in systemic blood vessels of preeclamptic women, in part because of increased levels in infiltrating neutrophils. We previously showed that MMP-1 increases vascular tone. The factors responsible for increased MMP expression in infiltrating neutrophils have not been identified. In this study, we explored the possible contributions of epigenetic mechanisms. We examined DNA methylation and gene expression in omental arteries of preeclamptic women, and determined whether DNA hypomethylation results in increased expression of the *MMP1* and *MMP8* genes in a neutrophil-like cell line (HL-60).

Friday

Methods: DNA was extracted from omental fat arteries of 5 normal pregnant and 7 preeclamptic women. The DNA was bisulfite treated and analyzed for DNA methylation using the Illumina HumanMethylation27 BeadChip. HL-60 cells were treated with 10 μM 5-aza-2-deoxycytidine (5-Aza), an inhibitor of DNA methylation, for 48 h, 0.01 μM PMA, an activator of protein kinase C, for 24 h or 5-Aza + PMA. MMP-1 and MMP-8 expression was assessed by qRT-PCR and Western blotting.
Results: The *MMP1* and *MMP8* genes were both significantly hypomethylated in preeclamptic omental arteries ($\Delta\beta$=-0.08, p=0.015 and $\Delta\beta$=-0.075, p=0.025, respectively). Treatment of HL-60 cells with 5-Aza significantly increased *MMP1* and *MMP8* gene expression compared to controls (36.5 ± 9.4-fold, p<0.001 and 3.2 ± 0.2-fold, p<0.001, respectively). PMA treatment increased *MMP1* gene expression (19.6 ± 3.3-fold, p<0.001), but not *MMP8*. PMA + 5-Aza resulted in a dramatic increase in *MMP1* expression compared to controls (2622 ± 167-fold, p<0.001), PMA alone (p<0.001) or 5-Aza alone (p<0.001). Western blot analysis of protein levels confirmed increased MMP expression.
Conclusions: The promoter regions of the *MMP1* and *MMP8* genes are hypomethylated in preeclamptic omental arteries. Experimentally induced hypomethylation increases expression of MMP-1 and MMP-8 in a neutrophil-like cell line.
Speculation: Epigenetic mechanisms (altered DNA methylation) contribute to the increased expression of MMP-1 and MMP-8 in systemic blood vessels of preeclamptic women by increasing *MMP1* and *MMP8* gene expression in infiltrating neutrophils. The elevated MMP levels may increase vascular tone and result in the hypertension associated with preeclampsia. NIH HL069851, P60MD002256

F-333

What Clinical Variables Predict the Onset of Cardiac Dysfucntion in Preeclampsia? Christy Pearce, Suzanne McGehee, Rebecca Epstein, Samantha Mast, Karen Playforth, Wendy Hansen, John O'Brien. *OB/GYN, University of Kentucky, Lexington, KY, USA.*
Background: Preeclampsia affects 5% of pregnancies and can be associated with transient cardiac dysfunction in the peripartum period. Current diagnostic criteria do not predict severity or incidence of adverse maternal outcomes including cardiac dysfunction. We sought to determine what clinical variables seen on admission may predict cardiac dysfunction associated with preeclampsia.
Methods: A retrospective case-control study of preeclamptic women admitted to our institution over a 2 year period. ICD-9 codes cross referenced with the echocardiography records identified charts for review. Patients diagnosed with preeclampsia who did not experience cardiac dysfunction were included as controls, and those with cardiac dysfunction diagnosed after preeclampsia were included as cases. Clinical variables recorded on the day of admission were compared. Statistical analysis included Student's t-test and Fisher's exact. A Bonferroni correction was used.
Results: 112 controls and 8 cases were identified. Clinical variables found to be strong predictors of cardiac dysfunction in the setting of preeclampsia included systolic blood pressure (SBP)> 160 mmHg, diastolic blood pressure (DBP)> 110 mmHg, mean arterial pressure (MAP)> 126 mmHg, the use of IV anti-hypertensives, chronic hypertension, uric acid>7.2 mg/dl, lactate dehydrogenase (LDH)> 234 U/L, and creatinine (CR)> 1 mg/dl (see figure 1).



Pulmonary edema, fetal growth restriction, abnormal umbilical artery dopplers, and liver transaminases were not found to be predictive. Mean values of uric acid (5.6 vs 7.1, p<0.001), LDH (189.7 vs 253.9. p=0.04), CR (0.7 vs 1.0, p<0.0001), DBP (93.8 vs 109.9, p=0.0001), SBP (158.9 vs 175, p=0.0142) were higher in those patients who developed cardiac dysfunction.
Discussion: Cardiac dysfunction in the setting of preeclampsia is associated with clinical variables that are indicative of cardiac strain, severity of disease, preexisting cardiac stress, or renal hypoperfusion. These results have been used to design a currently ongoing prospective cohort study examining this relationship.

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2-Methoxyestradiol (2-ME) Administration in Late Pregnancy Does Not Improve Fetal and Maternal Outcomes in a Mouse Model of Preeclampsia (PE). Rajan Poudel,[1] Joanna L Stanley,[1,2] Christian F Reuda-Clausen,[1] Xia Xu,[3] Timothy D Veenstra,[3] Colin P Sibley,[1,2] Sandra T Davidge,[1] Philip N Baker.[1,2] *[1]Departments of Obstetrics/Gynecology and Physiology, University of Alberta, Canada; [2]Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom; [3]SAIC-Frederick Inc, National Cancer Institute, USA.*
Background
Plasma concentrations of 2-ME and catechol-O-methyltransferase (COMT) are elevated in normal pregnancy but reduced in women with PE. Women with PE also exhibit decreased uteroplacental blood flow. Pregnant mice that lack COMT (COMT-/-) cannot produce 2-ME and are reported(1) to exhibit many features of PE including raised maternal blood pressure (BP), proteinuria and fetal growth restriction. 2-ME administration in COMT-/- mice leads to amelioration of the PE like phenotype(1). We hypothesized that uterine artery blood flow velocity is decreased in COMT-/- mice and would be improved by exogenous 2-ME administration.
Methods
Pregnant COMT-/- and control mice (C57BL/6) were injected subcutaneously daily with 10 ng of 2-ME or vehicle (olive oil) from gestational day (d) 12.5 to 17.5. BP was measured on d10.5 and 17.5. Proteinuria, uterine artery blood flow velocity and fetal weights were measured at d18.5. Serum 2-ME concentration at d18.5 was measured by liquid chromatography-mass spectroscopy. All data is presented as mean±SEM and were analyzed using student unpaired t-test.
Results
Administration of 2-ME increased circulating levels of this molecule in COMT-/- mice (0.03±0.01 vs 0.13±0.01 pg/0.05ml, p=0.01) to levels above those found in controls mice (0.09±0.02 pg/0.05ml; p=0.04). There was no difference in uterine artery blood flow velocity between COMT-/- and control mice before or after treatment. Contrary to previous report (1), COMT-/- mice exhibited a significant increase in systolic BP on d10 (118±2 vs 127±3 mmHg; p=0.002) but not at d17.5 (123±5 vs 119±7 mmHg; p=0.2). Interestingly, 2-ME administration did not have any effect on BP (125 ± 8 vs 125 ± 8 mmHg, p=0.95), proteinuria or fetal weight (1.10±0.02 vs 1.03±0.01, p=0.01) of COMT-/- mice.
Conclusion
COMT-/- mice did not exhibit changes in uterine artery flow velocity. Despite surpassing the physiological 2-ME levels, our intervention did not improve the phenotype observed in COMT-/- mice. Our studies in this mouse model provide no evidence to support the administration of 2-ME as a therapy for PE.
Reference
1.Kanasaki et al. Nature 2008.

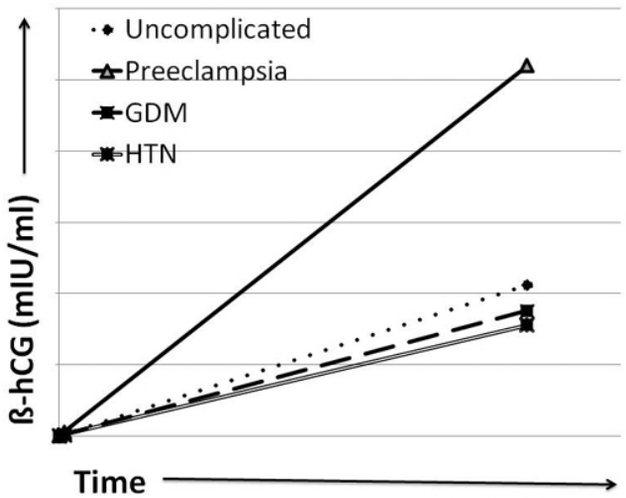
F-335

Placental Mitochondrial Function in Women with Preeclampsia. Laura M Reyes,[1] Ronald G Garcia,[2] Elvia S Diaz,[2] Sandra T Davidge,[1] Patricio Lopez-Jaramillo.[3,4] *[1]Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada; [2]Department of Research, Fundación Cardiovascular de Colombia, Floridablanca, Santander, Colombia; [3]Direction of Research, Fundación Oftalmológica de Santander-Clínica Carlos Ardila Lulle, Floridablanca, Santander, Colombia; [4]Direction of Research, Universidad de Santander, Bucaramanga, Santander, Colombia.*
Background
It has been demonstrated that women with preeclampsia (PE) from developing countries have an increased systemic inflammatory state and an up-regulation of syncytiotrophoblast expression of endothelial and inducible nitric oxide synthase (eNOS, iNOS). Additionally, eNOS down-regulation is associated with decreased mitochondrial number and function in other tissues. Therefore, we hypothesized that increase in syncytiotrophoblast eNOS/iNOS expression would be associated with increased placental mitochondrial expression/function and subsequently increased oxidative stress, thereby exacerbating placental complications of PE.
Methods
A blood sample was collected from 10 women with PE and 10 healthy pregnant women to determine maternal serum concentrations of TNF-α, IL-1β, IL-2 and IL-6. Placental eNOS/iNOS expression and nitrotyrosine levels (marker

of oxidative stress) were determined by immunofluorescence; cytochrome C oxidase (COX IV) expression was determined by western blot and its activity was determined by a microplate assay kit.
Results
Women with PE had higher serum concentrations of IL-6 than healthy pregnant controls (p=0.03). Placental eNOS (p=0.01), nitrotyrosine (p=0.002) and COX IV expression (p=0.02) were higher in women with PE. Whereas placental iNOS expression (p=0.15) and COX IV activity (p=0.8) were not different between the groups. A correlation between IL-6 and nitrotyrosine levels was found (p=0.02; r2=0.73).
Conclusions
Conflicting evidence regarding placental mitochondrial function in women with PE has been attributed to differences in the populations studied. In our study, women with PE had increased levels of IL-6 along with increased eNOS expression and evidence for oxidative stress. However, contrary to our hypothesis, no differences in placental COX IV activity between the groups were found, despite its increased expression in women with PE. Thus although mitochondrial expression is increased, the specific role for mitochondrial perturbations in PE needs further exploration.
Supported by Colciencias Grant # 656640820400

F-336

Rise of ß-hCG in the Early First Trimester Predicts Preeclampsia. Jared C Robins, Stephanie Lai, Beth J Plante, Bala Bhagavath, Sandra A Carson. *Obstetrics and Gynecology, The Warren Alpert School of Medicine at Brown University, Providence, RI, USA.*
Objective: The serum concentration of ß-hCG, a trophoblast cell derived hormone, reflects placental implantation and invasion. We hypothesized that the rate ß-hCG increase during early placentation predicts poor pregnancy outcome.
Design: Nested Cohort Study
Materials and Methods: Patients who had a live-birth after in vitro fertilization between January 2008 and January 2010 were evaluated for inclusion. Inclusion criteria were singleton implantation and live-birth. Demographic data included age, BMI, gravidity and parity. The ß-hCG concentrations on days 14, 16 and 23 post-embryo transfer were plotted. The rise of ß-hCG was calculated from the linear slope of the line. Patients with pregnancy outcomes of preeclampsia, gestational hypertension, and gestational diabetes were grouped. Mean slopes of ß-hCG were calculated for each pregnancy outcome group and compared with ANOVA.
Results: There were 330 live-births; 179 had complete data. Of these, 130 patients had uncomplicated pregnancies, 13 patients had preeclampsia, 23 patients had gestational hypertension, and 13 patients had gestational diabetes. The average change in ß-hCG in women who developed preeclampsia was 2600.8 mIU/ml/day, which was significantly greater than the average change in ß-hCG per day in women who did not have preeclampsia (uncomplicated: 1061.7 mIU/ml, gestational hypertension: 779.1 mIU/ml, gestational diabetes: 880.6 mIU/ml). Multiregression analysis determined there was no confounding effect from age, BMI or gravidity.
Conclusion: The rise of ß-hCG in patients destined to have preeclampsia is more than 2.5-fold greater than the rise in unaffected pregnancies. This further suggests that preeclampsia is a disease initiated in the first trimester at the time of placentation. Furthermore, ß-hCG may be a valuable marker to identify those patients at risk for preeclampsia. Early detection may enable us to heighten observation and intervention with these women at risk, possibly preventing the condition from progressing.



F-337

Preeclampsia (PE) or Chronic Kidney Disease (CKD): Are Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1) and Placental Growth Factor (PlGF) Suitable Biomarkers for Differential Diagnosis? A Rolfo,[1] R Attini,[1] AM Nuzzo,[1] V Consiglio,[2] F Fassio,[2] A Piazzese,[1] GB Piccoli,[2] T Todros.[1] *[1]Obstet. and Gynaecol., University of Turin, Turin, Italy; [2]SS Nephrology, S. Luigi Hospital, Orbassano, Italy.*
Objectives: The differential diagnosis between PE, maternal syndrome characterized by hypertension and proteinuria via kidney damage, and pregnant women with pre-existing kidney disease (CKD) is still a challenge. From a clinical point of view, hypertension due to undiagnosed CKD is often asymptomatic and may become evident only during pregnancy, thus being confused with PE. Importantly, despite PE and CKD share several clinical features, they have different pathogenesis and must be differentially managed. Several evidences indicate PlGF and sFlt-1, pro- and anti-angiogenic molecules altered during PE, as useful biomarkers for the early diagnosis of PE. The aim of the present study was to test if serological sFlt-1 and PlGF levels could also be useful to discriminate between PE and CKD, thus supporting clinical management.
Methods: Maternal serum samples were collected from PE (n=29), CKD (n=24) and physiological control (CTRL, n=38) pregnancies. sFlt-1 and PlGF serum levels were determined in parallel by specific and commercially available Electro Chemiluminescence Immunoassays (Elecsys, Roche, Germany) using an immunoanalyzer Cobas-e-411.
Results: sFlt-1 levels were significantly increased in PE relative to both CTRL (4.5 Fold increase, $p<0.01$) and CKD patients (5.6 Fold increase, $p<0.01$). No differences in sFlt-1 levels were found between CTRL and CKD patients. Moreover, we found significantly lower PlGF levels in PE relative to both CTRL (12.5 Fold decrease, $p<0.01$) and CKD (16.5 Fold decrease, $p<0.01$). No differences were found in PlGF levels between CTRL and CKD patients. We next investigated the sFlt-1/PlGF ratio, recently proposed parameter useful to discriminate between PE and normal pregnancy. sFlt-1/PlGF ratio was significantly increased in PE relative to CTRL (26.5 Fold Increase, $p<0.01$) and CKD pregnancies (25.4 Fold increase, $p<0.01$), while no differences were found between CKD and CTRL.
Conclusions: In the present study, we were able to discriminate between PE and CKD pregnancies by measuring sFlt-1/PlGF ratio in maternal serum samples. Our data strongly suggest sFlt-1/PlGF assay as a useful tool for PE vs CKD differential diagnosis, thus allowing proper clinical management. Further investigation is required to confirm the present findings.

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In Vitro Effect of Inositol Phosphoglycan P-Type on the Release of Preeclampsia–Related Vasoactive Factors by Human Trophoblast Cells. Federica Romani,[1] Anna Tropea,[1] Alessandra Familiari,[1] Elisa Scarinci,[1] Carola Palla,[1] Maria Letizia Uras,[1] Andrea Ciardulli,[1] Stefania Catino,[2] Antonio Lanzone,[2] Rosanna Apa.[1] *[1]Dipartimento per la Tutela della Salute della Donna e della Vita Nascente, Cattedra di Fisiopatologia della Riproduzione, Università Cattolica del S. Cuore, Roma, Italy; [2]Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina (EN), Italy; [3]Roma, Italia.*

In the recent years a family of putative insulin mediators, inositol phosphoglycans (IPGs), have been implicated in the pathophysiology of preeclampsia. Actually, high concentrations of bioactive IPG P-type (P-IPG) were found in human preeclamptic placenta and amniotic fluid. The high urinary levels of P-IPG reported in preeclampsia seem to increase few weeks before the clinical onset of this disease and to decrease after delivery. Although the causes for the preeclampsia related high levels of P-IPG remain to be clarified, possible paracrine effects of placental P-IPG have been suggested to play a role in the pathogenesis of this maternal syndrome.

In the present study we investigated whether D-chiro-inositol (glycan moiety of P-IPG) might affect placental secretion of soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin (sENG). Actually, these vasoactive factors are responsible for the correct placental development as well as for the remodelling of maternal vasculature during pregnancy. Moreover, growing evidences suggest a pivotal role for both sFlt-1 and sENG in the onset of preeclampsia.

Human placentas were collected at the time of elective caesarean sections at term of uncomplicated pregnancies from non smoking women with a history of normal blood pressure. After their purification, trophoblast cells were cultured with medium alone (control) or in presence of increasing concentrations of D-chiro-inositol (0.01, 0.1, 1, 10 μM). After 24h treatments sFlt-1 and sENG release were evaluated by ELISA.

In our in vitro system preliminary results show that D-chiro-inositol was able to significantly increase the release of both sFlt-1 and sENG. These data suggest could contribute to explain the placental dysfunction that lay behind preeclampsia onset.

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Pregnancy Outcomes after Unilateral Uterine Horn Ischemia Reperfusion in Rats: A Model of Preeclampsia, Fetal Growth Restriction or None of the above? Mauro H Schenone,[1] Wenyuan Zhao,[2] Giancarlo Mari,[1] Robert Ahokas.[1] *[1]Obstetrics and Gynecology, The University of Tennessee Health Science Center, Memphis, TN, USA; [2]Internal Medicine, The University of Tennessee Health Science Center, Memphis, TN, USA.*

Objective: It has been reported that a model of preeclampsia may be created in rats by using uterine ischemia reperfusion (IR) techniques. Our preliminary data have shown that 20 minutes of unilateral uterine IR in pregnant rats results in fetal demise. We aimed to study the effect of 10 minutes of unilateral uterine ischemia followed by reperfusion, on blood pressure, fetal growth, angiogenic and antiangiogenic factors.

Methods: Pregnant Sprague-Dawley rats were allocated to undergo laparotomy with unilateral uterine IR (experimental group, n= 8) or sham surgery (control group, n= 8). Surgery was performed on gestational day (GD) 14. Blood pressure (BP) was measured before the procedure and 6 days later. On GD 21 the rats were euthanized and mean fetal weight (MFW) and mean placental weights (MPW) were obtained. Serum was used to quantify levels of angiogenic and anti-angiogenic factors and 8-Isoprostane. Mann Whitney U test was used for statistical analysis. A $p < 0.05$ indicated statistical significance.

Results: The average change in the mean BP before and after the procedure was -16 mmHg (s= 20) and -1 mmHg (s= 18) in the intervention and control group, respectively (p= 0.197). The MFW was 4.29 g (s= 0.46) in the intervention group, and it was 4.48 g (s= 0.35) in the control group (p= 0.519). The MPW in the intervention group was 0.46 g (s= 0.07) and similarly 0.46 g (s= 0.04) in the control group (p= 0.439). The placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFLT-1) levels were undetectable in both groups, whereas the mean concentration of 8-Isoprostane was 2.5 ng/ml (s= 0.8) in the intervention group, and it was 2.9 ng/ml (s= 2.5) in the control group (p= 0.606).

Conclusion: Our data suggest that 10 minutes of uterine unilateral IR does not increase maternal blood pressure, and it does not affect the levels of angiogenic and antiangiogenic factors. There was a trend towards lower fetal weight in the experimental group. More data are needed to determine whether this model may cause fetal growth restriction.

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Characterization of Monocyte Subsets during Healthy Pregnancy and Preeclampsia in Humans and Rats. Floor Spaans,[1] Barbro N Melgert,[1] Theo Borghuis,[1] Pieter A Klok,[1] Bart Groen,[2] Marielle G van Pampus,[2] Monica Wong,[2] Winston W Bakker,[1] Marijke M Faas.[1] *[1]Path. and Med. Biology, University Medical Center Groningen; [2]Obstet. and Gynecol., University Medical Center Groningen.*

Introduction: Normal pregnancy is characterized by monocyte activation, which is exacerbated in preeclampsia (PE). In humans, the $CD14^+CD16^-$ (classical) and the $CD14^+CD16^+$ (non-classical) monocyte subset can be distinguished. In rats, similar subsets can be discriminated, the $CD172a^+CD43^{lo}$ (classical) and $CD172a^+CD43^{hi}$ (non-classical) subset. The non-classical subset has been associated with a more pro-inflammatory phenotype, and is increased in several inflammatory diseases. Our aim was to study monocyte subsets during pregnancy and PE in humans and in rats.

Methods: Blood samples from non-pregnant (n=18) women, PE patients (early onset; n=26) and gestational age-matched pregnant women (n=23) were labeled with α-CD14 and α-CD16 antibodies and processed for flow cytometry. Rats (female Wistar) were infused via a permanent jugular vein cannula with saline (n=7, control) or ATP (n=9, pro-inflammatory stimulus inducing PE-like symptoms such as proteinuria) on day 14 of pregnancy. Blood samples of day 13, 15, 17 and 20 were labeled with α-CD43 and α-CD172a antibodies. Monocyte subsets were analyzed by flow cytometry.

Results: Within the monocyte population, decreased percentages of classical monocytes were found in pregnant women (91,3±3,76;$p<0.01$) which were even further decreased in PE patients (87,7±6,83;$p<0.05$) compared to non-pregnant women (94,4±1,83). On the other hand, the percentages of non-classical monocytes in pregnant (8,68±3,76;$p<0.05$) and in preeclamptic women (12,2±6,83;$p<0.05$ vs. pregnancy) were increased as compared to non-pregnant women (5,65±1,86). In rats, we found no differences in the classical monocytes, but increased numbers of non-classical monocytes in pregnant vs. non-pregnant rats ($p<0.001$). ATP infusion increased the percentage of non-classical monocytes in pregnant but not in non-pregnant rats ($p<0.05$).

Conclusion: During pregnancy in humans and in rats the percentage of the pro-inflammatory non-classical monocytes increased, which is in line with the pro-inflammatory status of normal pregnancy. During PE this subsets is even further increased. The observation that induction of PE-like symptoms by ATP in pregnant rats significantly enhances the non-classical subset, may suggest that this subsets plays a role in the pathophysiology of PE.

F-341

Association between C1114G Polymorphism in Regulators of G-Protein Signaling RGS2 and Risk for Preeclampsia in the HUNT Cohort. Anne Stine Kvehaugen,[1] Oddgeir Holmen,[2] Oyvind Melien,[3] Anne Cathrine Staff.[1] *[1]Dept of Obstetrics and Gynecology, Oslo University Hospital and University of Oslo, Oslo, Norway; [2]HUNT Research Centre, Norwegian University of Science and Technology, Levanger, Norway; [3]Medicinal Products, Norwegian Directorate of Health, Norway.*

BACKGROUND: Preeclampsia (PE) is a hypertensive disorder of pregnancy with augmented mortality and morbidity for mother and offspring, with increased risk of cardiovascular disease later in life. The pathogenesis is incompletely understood, but is probably multifactorial. Heterotrimeric G proteins play key roles in the signalling pathways downstream of G protein-coupled receptors and mutations in these proteins are involved in a variety of diseases. The RGS2 (Regulator of G-protein signaling 2) subtype of the internal G protein regulators (RGS), is reported to be involved in regulation of weight and blood pressure.

OBJECTIVE: To assess whether women that develop PE differ from women without PE pregnancies regarding single nucleotide polymorphism (SNPs) of 3 G-protein receptor pathway associated genes.

METHODS: We assessed SNPs in a large Norwegian population-based biobank (the HUNT study) for 3 genes; the C825T polymorphism of the G-beta3-subunit of G-proteins, the A1166C polymorphism of the angiotensin II receptor and the C1114G polymorphism of RGS2. We studied 1237 PE cases and 2458 women with uncomplicated pregnancies.

RESULTS:

No significant differences were observed between the PE group and the control group regarding the frequency of the C allele of the A1166C polymorphism, the T allele of the C825T polymorphism or the G allele of the C1114G polymorphism.

A significant association was found between the G allele of the C1114G polymorphism and risk of PE when restricting the analysis to late onset PE (delivery either ≥37 or ≥ 34 weeks) vs. controls; OR for CG + GG vs. CC:

1.191 (95% CI: 1.031-1.376). For the early onset PE (delivery either < 37 weeks or <34 weeks), there was no significant association to PE development. DISCUSSION AND CONCLUSION: The C1114G Polymorphism in the Regulator of G protein signaling of subtype RGS2 has been linked to hypertension and increased BMI. Our study is the first to show that the G genotype is associated with an increased risk for late onset PE. This type of PE is viewed as 'maternal preeclampsia', caused by maternal factors, in contrast to 'placental preeclampsia' of early onset, with placental dysfunction and fetal growth restriction.

F-342

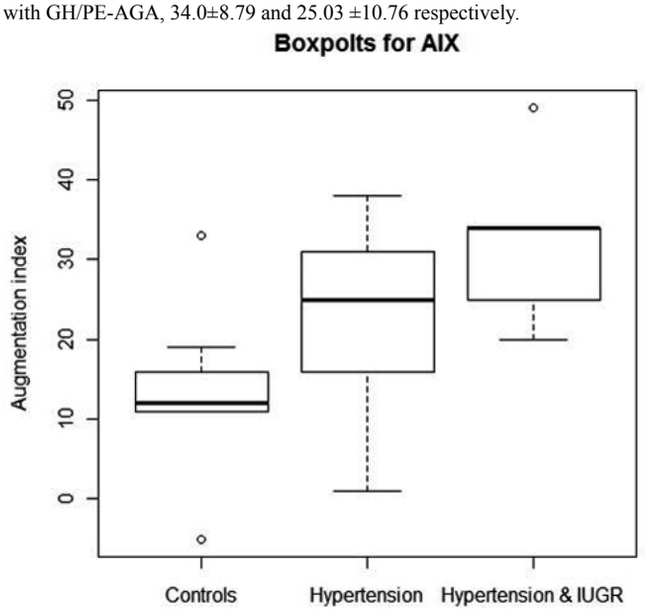
Pulse Wave Analysis in Gestational Hypertensive Disorders of Placental and Maternal Origin. T Stampalija, D Casati, C Zanardi, E Rosti, V Signorelli, C Mastroianni, D Dimartino, S Zullino, G Casu, E Ferrazzi. *Obstetrics and Gynecology, Childrens' Hospital Buzzi, University of Milan, Italy.*

Background: The main reason of failure of prevention, screening or diagnostic programs in gestational hypertensive disorders is probably due to inadequate classification based on time and/or severity domain. New evidences address the possibility of major stratification between placental and maternal origin. The former, typically determined by insufficient trophoblastic invasion, is associated with fetal growth restriction (IUGR).

Aim: The aim of our pilot study was to compare pulse pressure waveforms, as proxy of arterial stiffness, in pregnancies complicated by gestational hypertension/preeclampsia (GH/PE) associated with IUGR and GH/PE with appropriate for gestational age (AGA) fetuses.

Materials and methods: Augmentation index (AIx) obtained by radial applanation tonometry was performed in 34 women:18 at admission for GH/PE-AGA, 9 for GH/PE-IUGR and 7 controls with uneventful pregnancy. Differences between groups were evaluated by boxplot. Statistical significance was not evaluated due to the smallness of cohort.

Results: besides the control group (median AIx 12.00±11.87), the graph highlights the difference in AIx between women with GH/PE-IUGR and women with GH/PE-AGA, 34.0±8.79 and 25.03 ±10.76 respectively.



Graph 1. Comparison of AIx between normotensive women, GH/PE-AGA and GH/PE-IUGR by boxplot.

Conclusions: As expected, pregnant women with high blood pressure showed higher Augmentation index than normotensive pregnancies. Despite the small cohort, our pilot findings suggest that there exists a significant difference between gestational hypertensive disorders with adequately developed fetuses and gestational hypertensive disorders associated with IUGR fetuses. This adds genuine new evidence on the different pathophisiological origin of gestational hypertensive disorders, placental and maternal respectively. Understanding of underlying origin could have implication on prevention, clinical diagnosis and management.

F-343

The Number and Location of Vessels with Decidual Vasculopathy Correlate with Disease Severity in Preeclampsia. Droima Stevens,[1] Salwan Al-Nasiry,[1] Hans Bulten,[2] Marc Spaanderman.[1] *[1]Obstetrics & Gynaecology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Pathology, Radboud University Nijmegen Medical Centre, Netherlands.*

Objective: Preeclampsia (PE) is a disease associated with serious maternal and neonatal morbidity. In PE, decidual vasculopathy (DV), or acute atherosis, of spiral arteries was related to worse clinical outcome in PE. Our aim was to study the impact of individual histological features of DV on severity of PE.

Methods: Placental sections from 79 cases with PE were reanalyzed, scoring total number of vessels with DV, i.e. fibrinoid necrosis (tnDV), vessels with DV in decidua basalis (DB) and parietalis, and vessels with foam and perivascular inflammatory cells and thrombosis . Results were correlated with clinical and placental markers, using Spearman's rho and Mann-Whitney. P <0.05 was significant.

Results: Background parameters of age, BMI, use of tobacco, antihypertensives and MgSO4 did not differ between groups. tnDV correlated with higher diastolic blood pressure (p=0,022) and urine protein to creatinine ratio (p=0,040), shorter gestational age (p=0,025), lower birth weight (p=0,032), 5 minute APGAR score (p=0,014) and umbilical artery pH (p=0,001) and increased hematoma formation (p=0,037) and accelerated villous maturity (p=0,037). DB correlated with higher diastolic blood pressure (p=0,001), shorter gestational age (p=0,003), lower birth weight (p=0,009) and 5 minute APGAR score (p=0,008), decreased neonatal survival (p=0,002) and increased infarction (p=0,010).

Conclusions: In PE, tnDV and DB correlated with worse clinical outcome and increased placental pathology. This could signify a direct effect of DV on disease severity, with each lesion further aggravating spiral artery dysfunction and thus placental and clinical disease. The number of vessels with foam and inflammatory cells and with DV in decidua parietalis did not increase correlation with clinical and placental parameters, suggesting their association with disease severity is limited. It is unclear whether DV is the result of same disease process as PE or is caused by a predisposing underlying pathology. Studying DV could improve our understanding of the pathophysiology of PE and potentially lead to more targeted treatment options.

F-344

Soluble Prorenin-Receptor in Preeclampsia and Diabetic Pregnancies. Meryam Sugulle,[1] Harald Heidecke,[2] AH Jan Danser,[3] Ralf Dechend,[4,5] Dominik N Mueller,[5,6] Ulrike Maschke,[5] Genevieve Nguyen,[7] Anne Cathrine Staff.[1] *[1]Department of Obstetrics and Gynaecology, Oslo University Hospital and Faculty of Medicine, University of Oslo, Oslo, Norway; [2]CellTrend, Luckenwalde, Germany; [3]Division of Pharmacology, Vascular and Metabolic Diseases, Erasmus MC, Rotterdam, Netherlands; [4]HELIOS, Clinic, Berlin, Germany; [5]Experimental and Clinical Research Center, Charité Medical Faculty, Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; [6]University of Erlangen, Nikolaus-Fiebiger Ctr, Erlangen, Germany; [7]INSERM, Unit 1050, Collège de France, Paris, France.*

BACKGROUND: Pregnant women with preexisting diabetes mellitus (DM) or gestational DM (GDM) have an up to 5.6-fold increased risk for developing preeclampsia (PE). In PE, abnormal regulation of placental renin-angiotensin system (RAS) has been suggested, but the role of circulating RAS is less well understood. A soluble form of the prorenin receptor (sPRR) is found in the circulation and is able to bind prorenin, the inactive proenzyme form of renin. Prorenin bound to the sPRR has enzymatic activity. Diabetes and pregnancy are both characterized by very high prorenin levels and RAS activation, but the concentration of sPRR in both situations is unknown. We hypothesized that an augmented concentration of sPRR could contribute to the augmented Angiotensin-II sensitivity seen in preeclamptic women.

OBJECTIVE: To measure sPRR, using a newly developed ELISA, in preeclampsia and diabetic pregnancies, as compared to uncomplicated pregnancies.

STUDY DESIGN: Third trimester serum and citrate plasma from women with PE (n=81), DM (type 1, n=51; type 2, n=10; GDM, n=49), preeclampsia in DM (n=11) and 51 healthy pregnancies were analyzed for sPRR, renin and prorenin.

RESULTS: Prorenin levels were significantly increased in diabetic patients and renin levels decreased in preeclamptic patients compared to uncomplicated pregnancy, as expected. In preeclampsia, median sPPR concentration did not differ from the normal pregnant group. In contrast, circulating sPRR was significantly lower in pregnancies complicated by diabetes compared to healthy pregnancies.

CONCLUSION: Our results show that circulating sPRR is not dysregulated in preeclampsia, but reduced in pregnancies affected by diabetes mellitus.

Our hypothesis of an elevated sPRR in preeclampsia, potentially contributing to a higher prorenin/renin activity in preeclampsia, could not be confirmed in this study.

F-345

Prevalence of Cardiovascular Disease Risk Factors among Women with a History of Placental Abruption. Jan HW Veerbeek, Janine G Smit, Maria PH Koster, Steven V Koenen, Louis L Peeters, Bas B van Rijn, Arie Franx. *Division Woman and Baby, University Medical Centre Utrecht, Utrecht, Netherlands.*

Objective

Several studies have shown that the risk of premature cardiovascular disease (CVD) is increased after maternal placental syndromes (MPS), including placental abruption. Up to now CVD risk factors have been extensively studied in women with a history of preeclampsia but not in women who experienced placental abruption in a recent pregnancy. The aim of this study was to investigate the prevalence of CVD risk factors in this specific group of women.

Study Design

We performed a prospective cohort study of 75 women with a history of placental abruption and compared data to a reference group of 79 women with only uncomplicated pregnancies. Cardiovascular risk factors measured 6-9 months after delivery were: blood pressure, body-mass index (BMI), fasting blood glucose levels, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hs-CRP. Statistical analysis was performed using the independent samples T-test and chi-squared test. Data were further stratified for women with or without additional MPS-related complications, i.e. preeclampsia, gestational hypertension and intrauterine growth restriction.

Results

Placental abruption was significantly associated with increased systolic and diastolic blood pressure, BMI, fasting blood glucose levels, log hs-CRP, total cholesterol and LDL-cholesterol (figure 1). In women without additional MPS the associations remained significant for plasma lipid profile, BMI and fasting blood glucose levels, but not for diastolic and systolic blood pressure.

Baseline and outcome characteristics

	Placental Abruption (n=75)	Reference Group (n=75)	P-value
Patient characteristics			
Age	30,9 (4,9)	33,1 (4,4)	0.003
BMI, kg/m^2	25,4 (4,6)	22,8 (2,9)	<0,001
Nulliparity, n (%)	58 (77,3)	47 (59,5)	0.03
Chronic hypertension, n (%)	0 (0)	0 (0)	-
Diabetes mellitus, n (%)	0 (0)	0 (0)	-
Current smoker, n (%)	18 (24,0)	14 (17,7)	0.89
Characteristics of pregnancy			
Gestational age at delivery, weeks	29,6 (4,3)	40,3 (1,1)	<0,001
Infant's birth weight	1202 (698)	3624 (473)	<0,001
Small-for-gestational age, n (%)	22 (29,3)	1 (1,3)	-
Gestational hypertension, n (%)	11 (14,7)	0 (0)	-
Pre-eclampsia, n (%)	31 (41,3)	0 (0)	-
HELLP syndrome, n (%)	14 (18,7)	0 (0)	-
Gestational diabetes, n (%)	0 (0)	0 (0)	-
Cardiovascular risk factors			
Blood pressure, mmHg			
Systolic	122 (13,1)	114 (12,7)	0.002
Diastolic	79 (11,4)	75 (6,8)	0.05
Fasting blood glucose, mmol/l	5,1 (0,35)	3,9 (1,4)	<0,001
Hs CRP (Ln)	-0,79 (1,08)	0,88 (1,09)	<0,001
Plasma lipid profile			
Total cholesterol, mg/dl	194 (36,6)	144 (55,7)	<0,001
HDL-cholesterol, mg/dl	56 (15,1)	48 (15,0)	0.002
LDL-cholesterol, mg/dl	117,6 (35,7)	83,4 (43,2)	<0,001
Triglycerides, mg/dl	105,9 (60,6)	95,9 (72,6)	0.38
Tot. Chol/HDL-chol ratio	3,73 (1,25)	3,46 (2,44)	0.42

Data are given as mean±SD, n: number of participants.

Conclusions

A history of placental abruption is independently associated with increased BMI, fasting blood glucose levels, total cholesterol and LDL-cholesterol postpartum. Most likely these risk factors for CVC are already present before pregnancy, and we speculate that they predispose to placental abruption.

F-346

Prognostic Model of Markers Early in Pregnancy for Estimation of Cardiovascular Risk 6 Weeks after Pregnancy Complicated by Hypertension. Sanne Visser,[1,2] W Hermes, M Pampus van, K Boemenkamp, J Post van der, M Porath, C Koopmans, B Mol, C Groot de. *[1]Gynaecology, VUmc; [2]Gynaecologie, MCH.*

Recently increased epidemiologic evidence is available concerning the association of pregnancy complicated by hypertension and increased risk of cardiovascular disease (CVD) later in life. In this study we analyzed patients characteristics in early pregnancy to develop a prognostic model for CVD after pregnancy.

Women with hypertension six weeks after a pregnancy complicated by hypertension were selected from HYRAS study (n=306); follow up study of HYPITAT study. Variables analyzed include preeclampsia(PE) or pregnancy induced hypertension(PIH), maternal age, parity, smoking, family history and BMI and blood pressure(BP) at pregnancy intake. SPSS multivariable logistic regression analysis with a step backward selection procedure of predictors was preformed.

187 women were analyzed for blood pressure six weeks post partum, 75 (40%) had hypertension and 112 were normotensive. Hypertension was defined systolic BP≥140 mmHg, diastolic BP≥90 mmHg or antihypertensive medication use. Women had a greater risk on being hypertensive six weeks post after PIH vs PE (20% vs 54%). The mean systolic and diastolic BP at pregnancy intake was higher in hypertensive cases (resp 122mmHg vs 120mmHg and 75 mmHg vs 72 mmHg).

Advanced maternal age, multiparity and positive family history on hypertensive disease are significant predictive factors on hypertension six weeks post partum.

Characteristics	OR	95% CI
Preeclampsia vs Pregnancy Induced Hypertension	-0.780	-.266 - .115
Maternal age (yrs)	2.050	.001 - .031
Parity (Nulliparous)	1.592	-.037 - .339
Smoking	.515	-.170 - .290
BMI (≥ 25 kg/m2)	-.611	-.021 - .011
Blood pressure, systolic	1.016	-.004 - .012
Blood pressure, diastolic	0.234	-.010 - .013
Family history, hypertension	3.750	.182 – .590
Family history, cardiac event or stroke	-.794	-.231 – .097
Family history, hypertension in pregnancy	-.794	-.268 - .115

Table 1. Multivariate regression analyses on predictors of hypertension six weeks after pregnancy complicated by hypertension

Women who had a pregnancy complicated by hypertension with advanced maternal age, multiparity and positive family history for hypertensive disease are at risk for remaining hypertensive post partum. These data might suggest a preexisting state of hypertension before pregnancy and might be useful in prediction and follow up of CVD later in life.

F-347

Capabilities and Differences in Endothelium and Neutrophil Productions of IL-6, sIL-6R, and sgp130 between Normal Pregnancy and Preeclampsia. Yuping Wang, Shuang Zhao, Yang Gu, Lynn J Groome. *Ob/Gyn, LSU Health Sciences Center, Shreveport, LA, USA.*

Objective: IL-6 is an inflammatory cytokine. Increased maternal IL-6 and soluble gp130 (sgp130) levels and increased sgp130-to-sIL-6R/IL-6 ratio in preeclampsia (PE) suggest that increased IL-6 and sgp130 and/or decreased sIL-6R production comprise the event of increased inflammatory response in PE. This study was undertaken to determine the difference and ability of endothelial cells (ECs) and neutrophils in productions of IL-6, sIL-6R, and sgp130 in normal pregnancy and PE.

Methods: ECs were isolated from umbilical cords from normal and PE placentas. Neutrophils were isolated from freshly obtained maternal blood from normal and PE pregnant women. Confluent ECs (5 x105 cells/well) and freshly isolated neutrophils (2 x106 cells/well) were cultured separately or in combination (co-culture). Medium levels of IL-6, sIL-6R, and sgp130 were measured by ELISA. Since sIL-6R was mainly produced by neutrophils, we then determined if factors released by the placenta could promote neutrophil production of IL-6, sIL-6R, and sgp130. Neutrophils were primed by placental conditioned medium (CM). Data are expressed as mean ± S.E. and analyzed by t-test or ANOVA. A $p < 0.05$ was set as statistically significant.

Results: 1) Under non-stimulated condition: IL-6 and sgp130 were mainly produced by ECs. PE-ECs produced more IL-6 and sgp130 than normal-ECs, $p<0.05$; 2) sIL-6R was mainly produced by neutrophils and neutrophil productions of IL-6 and sgp130 were almost undetectable; Neutrophils from PE produced less sIL-6R than that from normal pregnant women, $p<0.05$; 3) Productions of IL-6, sIL-6R, and sgp130 were significantly increased when ECs were co-cultured with neutrophils, $p<0.01$; and 4) Neutrophils produced more IL-6 but less sIL-6R after primed by PE-CM, $p<0.05$.

Conclusions: ECs are major sources of IL-6 and sgp130, and neutrophils are a major source of sIL-6R. Increased interaction/adhesion of neutrophils to ECs promotes IL-6, sIL-6R and sgp130 productions. Placental derived factors could modulate neutrophil productions of IL-6 and sIL-6R. These results indicate different capability between ECs and neutrophils in IL-6, sIL-6R, and sgp130 productions and provide further evidence that the placenta plays a role in the regulation of neutrophil and EC function during pregnancy.

F-348

Loss of Specific Slit Protein Nephrin Is Associated with Reduced Antioxidant Superoxide Dismutase (SOD) Expression in Kidney Podocytes Shed in Women with Preeclampsia. Shuang Zhao, Susan Loyd, Lynn J Groome, Yuping Wang. *Ob/Gyn, LSU Health Sciences Center, Shreveport, LA, USA.*

Objective: Nephrin is a specific foot process slit protein in kidney podocytes. PARD-3 and PARD-6 are polarity proteins that mediate cell polarity. Disturbed slit protein nephrin and polarity protein PARD-3 and PARD-6 expressions and distributions have been found in shed podocytes in preeclampsia (PE). This study is to investigate if increased oxidative stress contributes to the loss of nephrin in shed podocytes from women with PE.

Methods: Nephrin, PARD-3, and CuZn-SOD expressions and distributions were determined by immunofluorescent staining in shed podocytes from women with PE. The expression and distribution of CuZn-SOD was used as an indicator of podocyte antioxidant capacity. Comparisons were made in immortalized podocytes (AB 8/13 cells) with or without exposure to hypoxic mimetic agent cobalt chloride. By extended cultivations of shed podocytes, we further determined if disturbed podocyte function could be recovered in vitro, in which expressions and distributions of nephrin, podoplanin, and CuZn-SOD were determined by immunofluorescent staining. Cell images were captured by Apotome Observer and reconstructed with Axiovision software.

Results: In differentiated podocytes (AB 8/13), nephrin, PARD-3, and CuZn-SOD were co-localized and expressed in the foot process area. In contrast, nephrin, PARD-3, and CuZn-SOD expressions were markedly reduced or lost at the foot process area in shed podocytes from PE. When differentiated AB 8/13 cells were treated with cobalt chloride, the pattern of nephrin, PARD-3, and SOD expressions and distributions was similar to that seen in PE-shed podocytes. We further noticed that shed podocytes from PE proliferated and after extended cultivations these cells could differentiate. The differentiation process was associated with functional protein regeneration.

Conclusions: Nephrin is a key podocyte specific protein that forms the slit diaphragm. The findings of lack of nephrin and SOD expressions in shed podocytes from PE and the co-localization of nephrin and SOD in slit diaphragm of differentiated podocytes suggest that proper cellular antioxidant activity is required for maintaining the functional integrity of glomerular podocytes. The phenomenon of hypoxia-induced loss of nephrin and CuZn-SOD further suggests that increased oxidative stress could be a causative factor to induce podocyte injury in PE.

F-349

First Trimester Non-Anemic Iron Deficiency and Preeclampsia. JoAnna Wawrzycki, Emily Campito, Monique Ho. *Obstetrics & Gynecology, University of Rochester Medical Center, Rochester, NY, USA.*

BACKGROUND: In a rat model of non-anemic iron (Fe) deficiency in early pregnancy, fetal brain upregulates vascular endothelial growth factor if Fe availability is low, and cell differentiation is altered. If this occurs in the human placenta, it may explain the abnormal vascular remodeling of preeclampsia. The purpose of this study is to explore the potential association between maternal indicators of early subclinical Fe deficiency and preeclampsia.

METHODS: Retrospective cohort analysis. Birth registry data 2007–2010 and chart review identified women who developed preeclampsia, had a singleton, first trimester serum screening, and placental evaluation. Control subjects had no gestational hypertension. Women with first trimester hematocrit (Hct) <30%, abruption, clinical chorioamnionitis, anomalous fetus or known hematologic disorder were excluded. Placental staining was done on a subset of subjects for Fe and transferrin receptor (TfR) with Prussian Blue and immunohistochemistry, respectively. Results were compared using cell #/low power field for Fe, and subjective stain intensity score for TfR. Soluble TfR and ferritin levels in first trimester serum were determined by ELISA, and groups compared by t-test. Categorical variables were analyzed by Fisher's Exact test. Logistic regression was performed to control for covariates.

RESULTS: 63 preeclamptic women and 61 controls met study criteria. First trimester serum TfR levels (15.4 +/- 4.2 vs. 15.6+/- 5.8 nmol/L; p=0.85), ferritin (43.3 +/- 36.4, and 56.3+/- 40.0 ng/mL; p=0.06, and Hct (36.8% vs. 37.6%; p=0.13) did not differ between preeclamptics and controls. African American (AA) preeclamptic subjects had significantly lower first trimester serum Hct compared to non-AA subjects with preeclampsia (35.4 +/- 3.1, and 37.7 +/-2.7, respectively, p=0.004), and lower serum ferritin than AA controls (36.5+/- 28.7 vs. 68.3+/-38.6; p=.02). Regression did not demonstrate any interaction between variables, except for that expected between chronic hypertension and preeclampsia (OR 4.0; 95% CI 1.2-13.8). Fe and TfR staining in the placenta did not appear different between groups.

CONCLUSIONS: First trimester serum TfR, ferritin and hematocrit, and placental Fe and TfR staining do not in general differ between women who develop preeclampsia and those who do not. There is evidence that for a subset of women, i.e. the AA participants in our study, early Fe deficiency may have an association with the disease.

F-350

TNF-α Regulated sFlt-1 Level in Cultured Placental Explants Via MAPK Pathway. Yali Xiong,[1] Indhu M Prabhakaran,[1] Eliezer J Holtzman,[3] Stacey Jeronis,[2] Dan A Liebermann,[1] Barbara Hoffman,[1] Ossie Geifman-Holtzman.[4] *[1]Fels Institute, Temple University School of Medicine, Philadelphia, PA, USA; [2]OBGYN, Temple University School of Medicine, Philadelphia, PA, USA; [3]Nephrology and Hypertension Institute, Sheba Medical Center, Tel-Aviv University, Tel Hashomer, Israel; [4]OBGYN, Drexel University, Philadelphia, PA, USA.*

Objective: To disclose the activation of the MAPK signal cascade in response to preeclampsia associated stressor TNF-α in placental explants and to evaluate the causal link between MAPK pathway activation and sFlt-1 regulation in placental explants.

Study Design:

Fresh third trimester placenta explants were collected and set up for in vitro culture with 10%FBS RMPI at 37ºC and were treated with preeclampsia associated stressor TNF-α (20ug/mL for 10 min, 20 min, 24hr, 48 hr or 72 hr). Total protein was extracted from the explants and western blots were processed to detect the expression of stress response protein Gadd45a and the downstream MAPK signal effectors (i.e. phosphor-JNK and phosphor-p38). JNK and p38 inhibition were also carried out by using SP600125 (50uM) or SB204580 (10uM) prior to above TNF-α treatment. The supernatant of the cultured placental explants were collected simultaneously. Expressional levels of these stress response elements together with the MAPK signal pathway effectors were assessed via western blots and sFlt-1 levels were evaluated with Elisa Kit.

Results:

Gadd45a protein was induced together with the downstream phosphor-JNK and phosphor-p38 activation in response TNF-α. The activation of phosphor-JNK and phosphor-p38 were inhibited by SP600125 and SB203580 respectively. The up-regulation of sFlt-1 levels by TNF-α was depleted by phosphor-JNK inhibition but not phosphor-p38 inhibition.

Conclusions:

Our data using placental explants model provides evidence that TNF-α which was reported to be increased in preeclampsia activated stress sensor protein Gadd45a together with its downstream MAPK effectors phosphor-JNK and phosphor-p38. However, only activated JNK regulates sFlt-1 levels but not phosphor-p38 which was also activated upon Gadd45a induction. Further evidence will be obtained by in-vivo experiments using Gadd45a knockout mice.

F-351

Maternal Blood Flow and Oxygen Delivery in Sub-Groups of Preeclampsia. S Zamudio, NP Illsley, M Alvarez, A Al Khan. *Obstetrics & Gynecology, Hackensack University Medical Center, Hackensack, NJ.*

Preeclampsia (PE) without intrauterine growth restriction (IUGR) is often considered more benign than earlier onset PE with or without IUGR. This study was designed to measure maternal uterine artery blood flow and O_2 delivery in sub-categories of preeclampsia and to explore whether normalization to fetal or placental weight enhances explanatory power. **Methods:** Doppler and ultrasound was used to measure bilateral uterine artery diameters and blood flow velocity calculation of blood flow. Arterial O2 content was calculated from hgb concentration and multiplied by flow for O2 delivery. ANOVA with a Bonferroni correction was used to compare between groups (control [NL] n=122, late-onset PE [LPE] n = 28, early onset PE [EPE] n=13, PE+IUGR n=13, and IUGR without elevated BP n=7). Data are reported as mean±SD. **Results**: Abnormal unilateral or bilateral uterine artery Doppler resistance indices were present in 0% of NL, 50% of LPE, 73% of EPE, 85% of PE+IUGR and 0% IUGR (p<0.0001). Maternal uterine blood flow/kg fetal weight was lower in the IUGR

group only (NL 183±72, LPE 169±53, EPE 137±82, PE+IUGR 170±92, IUGR 64±40* ml/min/kg). In contrast, when normalized to placental weight, flow was reduced by >40% in EPE (NL 1.26±0.12, LPE 1.34±0.54, EPE 0.73±0.36*, PE+IUGR 1.18±0.74, IUGR 1.20±0.43 ml/min/g placenta). Maternal O_2 delivery was similar across all 5 groups when normalized to fetal weight (NL 31±8, LPE 29±9, EPE 26±17, PE+IUGR 31±7, IUGR 37±14, ml/min/kg fetus). However, again, normalization of maternal O_2 delivery to placental weight showed a reduction only in EPE (NL 0.21±003, LPE 0.23±0.09, EPE 0.14±0.07, PE+IUGR 0.21±0.09 and IUGR 0.22±0.07 ml/min/g). **Conclusions**: When blood flow data are normalized to fetal weight, there is little difference in more vs. less severe forms of preeclampsia. Normalization to placental rather than the fetal weight reveals the placental dysfunction present in EPE, appears to track the severity of PE and highlights the relatively benign presentation of LPE. Support: HD 042737, HD046982, TW007444

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NMDA Receptor 1 and 2B Expression Changes in Medial Preoptic Area Gonadotrophin-Releasing Hormone (GnRH) Neurons during the Rat Estrous Cycle. Beom Su Kim,[1] Mateen Haroon,[2] Biren Patel,[1] Glenna CL Bett,[1,3] Armando Arroyo.[1] *[1]Gynecology-0bstetrics, SUNY, University at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY, USA; [2]Neuroscience Program, SUNY, University at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY, USA; [3]Physiology and Biophysics, SUNY, University at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY, USA.*
Glutamate regulates hypothalamic gonadotropin-releasing hormone (GnRH) neuron excitability. It is well established that GnRH neurons express glutamate receptors including AMPA, kainate and NMDA receptors (NR). NR1, NR2A and NR2B are expressed in GnRH neurons. It is unknown whether NMDA receptor subunit expression changes during the estrous cycle. Here we investigate NR1, NR2A and NR2B expression in medial preoptic area GnRH neurons during diestrus, proestrus, and estrus in GnRH-GFP cycling rats (n=5 rat brains/stage) using single-label immunohistochemistry and confocal microscopy. NR1 expression increased in the afternoon during diestrus (25 vs 42%, p=0.03) and estrus (22 vs 44%, p=0.02), and no change expression during proestrus AM and PM. NR2A expression in GnRH neurons did not change during the estrous cycle. Expression of NR2B receptors increased during the estrous cycle, reaching the highest level of expression at proestrus PM (68%) and estrus AM (64%). This data supports the hypothesis that NR1 and NR2B expression in GnRH neurons changes during the rat estrous cycle. Since estrogen fluctuates during the estrous cycle it is possible that it regulates NR1 and NR2B expression in GnRH neurons.

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Serum Levels of Vitamin D in Lean and Obese Pregnant Women. VD Castracane,[1] B Martinez,[1] U Shah,[1] T Loi,[2] S Durham,[2] CG Maguire.[1] *[1]Obstetrics and Gynecology, Texas Tech University Health Sciences Center at the Permian Basin, Odessa, TX, USA; [2]Immunodiagnostic Systems, , Scottsdale, AZ, USA.*
OBJECTIVE: Obesity has become a major physiological problem with increasing indications of pregnancy involvement. Obesity has been associated with decreased Vitamin D levels, diabetes and reproductive risks. We present our preliminary data on 25-OH D levels through gestation in lean, obese and gestational diabetic subjects that test the hypothesis that Vitamin D IS decreased in obese pregnant women. Seasonality for Vitamin D may be related to greater sun exposure in the summer months.
METHODS: Normal pregnant women were enrolled in early pregnancy at 6-9 weeks GA who provided blood samples every two weeks through gestation. We report serum levels of Vitamin D in lean (n=8), obese (n=6) and a gestational diabetic pregnant subject. Subject enrollment for this study continues. A separate group of samples from singleton (n=35) and twin (n=31) pregnancies at 15-20 weeks of gestation and without regard to BMI, were analyzed for Vitamin D to determine whether increased placental mass (twins) would increase 25-OH D levels over singleton pregnancies, as an indication of a placental contribution to circulating levels. Vitamin D was measured by chemiluminescent immunoassay (Immunodiagnostic Systems). Data were compared with ANOVA.
RESULTS: . Mean (+/- SEM) Vitamin D levels for the lean subjects was 28.39 +/- 1.98 ng/ml and for the obese subjects was 19.80 +/- 3.05 ng/ml. and for the gestational diabetic subject was 17.44 ng/ml. Of six lean subjects, 5 had a two values or more above the sufficiency level for Vitamin D (>30 ng/ml) and only one subject in the obese or gestational diabetic subjects had a value >30.0 ng/ml. Most subjects, either lean or obese, had a slight increase in 25-OH D levels over the course of gestation. Levels of Vitamin D during the summer months were generally unchanged. In the singleton and twin comparison there were no differences in M+SE or in linear regression of Vitamin D levels against BMI
DISCUSSION: Levels of Vitamin D are higher in lean pregnant women than in obese and gestational diabetic pregnant women. Maternal serum levels of Vitamin D are generally insufficient in the obese group and increased in the lean group. Time of year did not significantly affect 25-OH D levels in the two subject groups. No difference in singleton and twin pregnancies was noted.

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The Effect of Fetal Number on Maternal Serum Leptin Levels in the Sheep. VD Castracane,[1] L Penrose,[2] A Carpenter,[2] S Prien,[2] RL Norman.[3] *[1]Obstetrics and Gynecology, Texas Tech University Health Sciences Centers, Odessa, TX, USA; [2]Obstetrics and Gynecology, Texas Tech University Health Sciences Centers, Lubbock, TX, USA; [3]Pharmacology and Neuroscience, Texas Tech University Health Sciences Centers, Lubbock, TX, USA.*
Background: The source of leptin in the maternal circulation during pregnancy remains controversial. Maternal adipose tissue remains a significant contributor but it has been suggested that the placenta, which is known to express the leptin gene, is another potential contributor. Studies to address this point generally involve perfusion of the postpartum placenta but "physiological" studies have not been done which support such a conclusion. We sought to do more physiological studies and in the rat and demonstrated that the adjustment of the number of placental implantation sites in early gestation resulted in changes in maternal serum leptin concentration, with the lowest levels in the full complement of fetuses (>10) and the greatest levels with the fewest number of fetuses (1-2 implants). In women we compared singleton and twin pregnancies and have observed that two placentas contribute no additional leptin to the maternal circulation and that in both singleton and twins the correlation with BMI is virtually identical, suggesting that maternal adipose tissue is the major, if not exclusive, source of maternal circulating leptin. In this study we examined this relationship in the pregnant ewe.
Methods: Sheep were mated with known fertile males and established timing of pregnancy by chinball marker. The number of fetuses was determined at delivery (singleton, n=4; twins, n=8; or triplets, n=4). Serial blood samples were obtained bi-weekly and serum frozen until assayed for leptin with a multispecies Leptin RIA (Millipore). Estrogen and progesterone were analyzed using commercial immune assays. Data were compared using Anova.
Results: Maternal serum leptin levels are slightly increased during gestation in singleton, twin and triplet groups but the leptin levels between groups was not significantly different.
Conclusion: In the pregnant sheep the results seem to be the same as in our studies in the rat and human; specifically that fetal number is without effect on maternal serum leptin levels. This picture suggests that the common physiological situation across species is that placental leptin remains in the placenta for local paracrine actions and peripheral levels originate in maternal adipose tissue.

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Modulation of Binding Properties of Estrogen and GnRH Receptors in Immortalized GnRH Neurons by Concomitant Treatment with Estrogen and GnRH. Rebecca J Chason,[1] Po Ki Leung,[2] James H Segars,[1] Lazar Z Krsmanovic,[2] Kevin J Catt.[2] *[1]Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; [2]Endocrinology and Reproduction Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.*
Background: Treatment of immortalized GnRH neurons (GT1-7) with estradiol (E2) and/or GnRH alters GnRH secretion, cAMP production and spontaneous action potential firing. Such changes contribute to the function of the HPA axis but are incompletely understood.
Objective: To investigate the receptor-mediated mechanisms by which E2 alters GnRH secretion in GT1-7 cells.
Materials and Methods: GT1-7 cells were cultured for two days in a 1:1 mixture of DMEM and F12 medium supplemented with 10% heat-inactivated fetal bovine serum. The medium was replaced by serum- and phenol red-free medium and 24-hours later saturation and displacement binding studies were performed on membrane fractions.
Results: Displacement binding studies exhibited specific, high-affinity binding of the radioiodinated GnRH agonist, des-Gly10[D-Ala6]GnRH N-ethylamide (125I-[D-Ala6]Ag), that was inhibited by GnRH in a dose-, time-, and temperature-dependent manner. When treated with GnRH and E2, the number

of GnRH-binding sites was significantly decreased from 467 ± 21.2 fmol/mg protein to 262.7 ± 42.7 fmol/mg protein with unchanged EC50 (Fig. 1). Saturation binding studies of the ER revealed a decrease in ER binding sites when treated with GnRH (Fig. 2). The binding properties of the ER were not changed in the presence of a GnRH antagonist analog, suggesting that activated GnRH-R may influence ER binding properties (Fig. 3.).

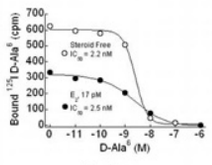

Figure 1

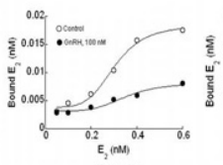

Figure 2

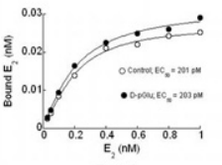

Figure 3

Conclusion:Binding properties of the ER and GnRH-R expressed in GT1-7 neurons were altered by concomitant treatment with E2 and GnRH, suggesting that agonist-induced interactions occur between the receptors with consequent changes in downstream signaling. These findings contribute to the understanding of the molecular mechanisms involved in regulation of the HPA axis.

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Whole Cellular Cytokine Composition of Decidualized Endometrial Stromal Cells Is Modified by Knock-Down of Syndecan-1 Followed by Imitation of Embryo Contact. Alexandra P Hess, Jan-Steffen Krussel, Daniel Fehr, Andrea Schanz, Wolfgang Janni, Dunja M Baston-Bust. *Department of OB/GYN and REI, University of Duesseldorf, Medical Center, Duesseldorf, Germany.*

Introduction:

The successful establishment of pregnancy in humans depends on a synchronisation of maternal and embryonic factors. Cytokines are well-characterized molecules in the peri-implantation period. IL-1beta was identified as an early secretion product in the supernatant of cultured embryos in-vitro and in early human pregnancy. These molecules play important roles in mediating the acceptance of the maternal immune system towards the semi-allograft embryo next to their angiogenic abilities. Besides the signalling through the classical G-protein coupled receptors, syndecans (Sdc) also take part in mediating chemokine function as co-receptors. Syndecans are localized on the cell-surface and in the extracellular matrix. They act multifunctionally in humans, are localized nearly ubiquitiously and are involved in tumour growth, immune cell function and angiogenesis.

In our opinion, Sdc-1 supports cytokine signalling in human decidualized endometrial stroma cells and supports a proper embryonic implantation.

Material and methods:

A stable and tetracycline (Tet) inducible human endometrial stroma (St-T1) Sdc-1 knock-down cell line (KdS1) was generated by transfection of Sdc-1 short hairpin RNAs under the control of a trans localized Tet repressor. St-T1 and KdS1 were decidualized (d) by cAMP and P4 and decidualization was verified by prolactin and IGFBP-1 PCR. Cytokine expression profiles were performed by dot blot analysis of whole cellular protein [50µg] of Tet-induced dKdS1 compared to dSt-T1 after 48h coincubation with IL-1ß [0,1ng/ml].

Results and conclusion:

The chemokines CXCL1, IL-6 and CXCL-8 were most significantly expressed in dKdS1 compared to dSt-T1. MIF was significantly higher expressed in dKdS1 versus dSt-T1. The cellular expression levels of CD154, IL-13 and PAI-1 were similar. Some chemokines and infection-associated molecules, as CCL2, CXCL11, G-CSF and GM-CSF, were expressed in dKdS1 exclusively. On the other side, the expression of IFN-γ was restricted to dSt-T1 cellular protein.

Regarding the imitation of embryo contact, Sdc-1 seems to play a regulatory role in endometrial stroma cells. The knock-down of this co-receptor of cytokines leads to a different expression pattern of proteins that might result in a dysregulated implantation.

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Regulation of Nuclear Hormone Receptor Expression in the Pituitary. Jonathan Kim, Weiming Zheng, Constance Grafer, Lisa Halvorson. *Department of Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA.*

BACKGROUND: Normal sexual differentiation and development depends on regulation and expression of gonadotropins from the anterior pituitary gland, a process that requires a complex array of transcription factors and associated DNA-regulatory elements. Previous studies from our group and others have demonstrated that the orphan nuclear hormone receptors, steroidogenic factor-1 (SF-1) and liver receptor homolog-1 (LRH 1), stimulate LHβ gene expression, whereas DAX-1, small heterodimer partner (SHP) and chicken ovalbumin upstream promoter transcription factor (COUP-TF) isoforms blunt expression. As little is known regarding hormonal regulation of the expression of these factors, we investigated expression across the rat estrous cycle and following treatment of dispersed primary pituitary cells with gonadotropin-releasing hormone (GnRH) or gonadal steroids.

METHODS: 1) Rat anterior pituitaries were collected at proestrus (PE0900, 1300, 1700, 2100), estrus (E0900), metestrus (ME0900) and diestrus (DE0900) and total RNA was obtained. 2) Male rat primary pituitary cells were dispersed, plated, and treated with GnRH 100nM x 6h, estradiol (E2) 100nM x 24h, or dihydrotestosterone (DHT) 100nM x 24h. qPCR was subsequently carried out to detect the expression of SF-1, LRH-1, DAX-1, SHP, and COUPTF-1/2 using Taqman gene assays. Statistical analysis was performed using the SigmaStat statistical software package with significance set at $p<0.05$.

RESULTS: 1) SF-1 transcript levels were approximately 50% lower at PE2100 relative to DE0900 ($p<0.05$). In contrast, expression of the related stimulatory factor, LRH-1, did not vary significantly across the cycle. Expression of the inhibitory factors, DAX1 and SHP, increased significantly by 200% and 500% respectively from E0900 to DE0900 ($p<0.05$). Although not reaching statistical significance, COUP-TF2 levels also increased over this time span while COUP-TF1 levels were unchanged. 2) GnRH and DHT treatment significantly decreased SF-1, DAX-1 and SHP expression by 30-60% with minimal effects on the other transcription factors ($p<0.05$). In preliminary experiments, the only impact of E2 treatment was a small increase in COUP-TF1/2 mRNA levels, but this did not reach statistical significance.

CONCLUSIONS: Our data suggest that orphan nuclear receptor expression in the pituitary gland is regulated in a complex pattern by hormonal factors, allowing for precise modulation of LHβ expression.

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Di (2-ethylhexyl) Phthalate (DEHP), a Common Medical Plasticizer, Activates the Pregnane X Receptor and Induces Human CYP3A4 Enzyme Expression and Activity In Vitro, Implications for Steroid Metabolism. Oumar Kuzbari,[1] John G Lamb,[2] Michael R Franklin,[2] Jessie Dorais,[1] C Matthew Peterson.[1] *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA; [2]Pharmacology and Toxicology, University of Utah, Salt Lake City, UT, USA.*

Background: Di (2-ethylhexyl) phthalate (DEHP) is an endocrine-disrupting chemical used in the manufacture of products made of flexible polyvinyl chloride, including medical bags and food packaging and has been reported to interact with the pregnane X receptor (PXR). Human CYP3A4 enzyme has an essential role in the hydroxylation of steroid hormones and is regulated at the molecular level by the PXR.

Objective: To determine the effect of DEHP on CYP3A4 transcription and enzyme activity in human hepatoma cells.

Materials and Methods: DPX2 cells, human hepatoma cells that are stably integrated with human PXR and a luciferase construct containing the CYP3A4 promoter, were exposed to DEHP at physiologically relevant concentrations in a 96 well plate. Activation of human PXR regulated gene expression was determined by luciferase activity (normalized to cell viability). Human CYP3A4 enzyme activity was determined using the luminescent CYP3A4 substrate (P450-Glo CYP3A4 Assay with luciferin isopropyl acetal). All assays were measured with a BioTek Synergy2 plate reader. DMSO served as control.

Results: DEHP (1-25 µg/ml,) caused a significant activation of the CYP3A4 promoter, via the PXR compared to control ($p<0.05$).

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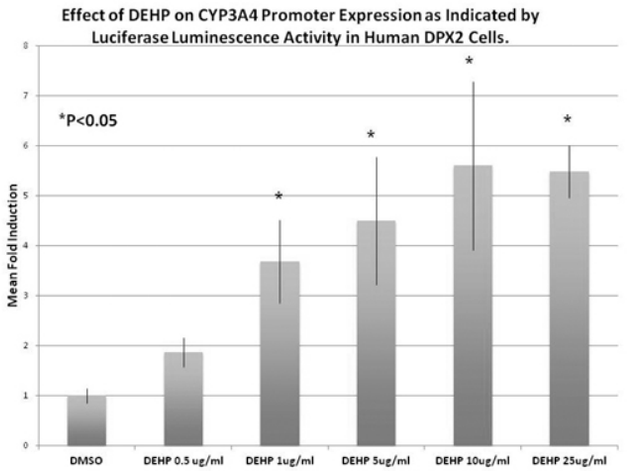
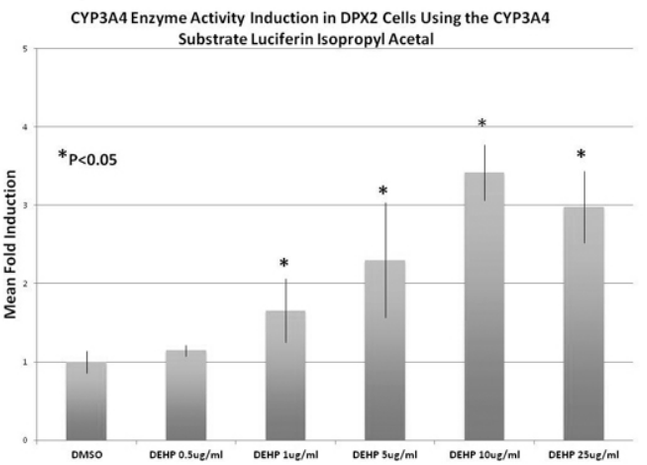


DEHP also induced CYP3A4 enzyme activity at concentrations relevant to human exposure.



Conclusion: DEHP, in concentrations relevant to human exposure, induces the CYP3A4 enzyme expression and activity in DPX2 hepatoma cells in vitro and may affect endocrine function by altering steroid hormone metabolism/action through a PXR mediated mechanism.

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Triclocarban Affects Circulating Cytokines, Chemokines and Acute Phase Proteins in Pre-Pubertal Girls. Lindsey A Loomba-Albrecht,[1] Nancy A Gee,[2] Mady Hornig,[3] Dennis M Styne,[1] Antoni J Duleba,[4] Bill L Lasley.[2] *[1]Department of Pediatrics, University of California Davis Medical Center, Sacramento, CA, USA; [2]Center for Health and the Environment, University of California, Davis, CA, USA; [3]Center for Infection and Immunity, Columbia University Mailman School of Public Health, New York, NY, USA; [4]Department of Obstetrics and Gynecology, University of California, Davis, CA, USA.*

Background: Triclocarban (TCC) is an antimicrobial additive in personal care products. TCC acts *in vitro* to augment the signal transduction of steroid hormones. TCC can be absorbed transdermally in humans.

Aim: To determine if transdermal TCC exposure from a normal shower with TCC-containing bar soap can have adverse effects in pre-pubertal girls.

Methods: Ten girls (8 - 11 years old) were monitored by collecting blood samples prior to and 1 and 3 hours following a shower using a commercial TCC-containing bar soap. Showers ranged from 3 - 10 minutes and the girls had BMIs that ranged from 25 - 57. Blood samples were evaluated for circulating TCC concentrations by LC-MS/MS and 48 cytokines, chemokines, and acute phase proteins (IL-1α2, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL12-p70, IL-13, IL-15, IL-17A, IL-17F, IL-1ra, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ, TGF-β, VCAM, ICAM-1, IP-10, RANTES, GM-CSF, MIG, MIP-1α, MIP-1β, MCP-1, MCP-3, VEGF, PAI-1, Leptin, ENA78, PDGF-BB, NGF, LIF, Eotaxin, FGF basic, TRAIL, GRO-α, HGF, sFas ligand, sCD40 ligand, Resistin, SCF, G-CSF, and M-CSF) were assessed by multiplexed, bead-based immunoassay (Luminex 3D).

Results: Circulating TCC levels ranged from 0.2 - 0.7 ng/mL prior to the shower, reaching 0.8 - 2.4 ng/mL and 1.0 - 71 ng/mL at 1 and 3 hours following the shower, respectively. There was no obvious relationship between concentration and shower time or subject BMI. Approximately one third of the 48 cytokines measured either increased or decreased following the shower. Subjects with the greatest percent increases in TCC levels from pre-shower baseline levels differed from subjects with the smallest degree of change in TCC levels in immune molecules associated with inflammatory cascades.

Conclusion: These results confirm that transdermal absorption of TCC from commercial bar soap during a normal shower routine can result in detectable.

Support: P42 ES04699

F-360

Levels of the Endocannabinoid 2 Arachidonylglycerol in Blood during the Menstrual Cycle and in Women with Threatened Miscarriage. Timothy H Marczylo, Amy Gorman, Sara-Jane Mason-Birks, Patricia MW Lam, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences Sections, CSMM, University of Leicester, Leicester, United Kingdom.*

Introduction: Regulation of the levels of the endocannabinoid arachidonoylethanolamide (anandamide, AEA) are associated with infertility, implantation, miscarriage, labour and gynaecological pathologies. The structurally related 2-arachidonylglycerol (2AG), though a more potent cannabinoid receptor agonist, has been little investigated because of difficulties associated with its analysis and stability. Here we show that silver ion chromatography with UHPLC-ESI-MS/MS is accurate and reproducible and yields unprecedented sensitivity. The method, combined with solid-phase extraction was employed to measure 2AG and related compounds in serum, plasma.

Methods: The methodology was validated according to FDA guidelines. This was a combination of two studies - a longitudinal study of 8 women in whom 2AG levels were measured through the menstrual cycle and a cross sectional study of 20 women with threatened miscarriage in whom blood levels of 2AG were also determined.

Results: LOD and LOQ were 1.7 and 4.2fmol on column. SPE recoveries were 55% and 61% for plasma and serum, respectively. Intra- and inter-day variability was minimal with CV values of 0.41 and 0.49%, respectively. Storage and freeze-thaw of samples had minimal effects. 2AG was elevated in serum compared to plasma. 2AG level was low in early follicular phase. Mean 2AG level in non-pregnant women was 4.75nM. In the pregnant women mean 2AG was 3.56nM and no significant differences were observed between asymptomatic women and those reporting pain or bleeding.

Conclusions: 2AG levels are tenfold greater than AEA and can be accurately and reproducibly measured in blood.

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Serum Kisspeptin, Inhibin B and Total Inhibin Concentrations in Patients with Functional Hypothalamic Amenorrhea. Blazej Meczekalski, Agnieszka Podfigurna-Stopa. *Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland.*

INTRODUCTION

Functional hypothalamic amenorrhea (FHA) is defined as nonorganic and reversible disorder, in which GnRH pulsatile secretion impairment plays a key role. There are main three types of FHA: stress-, weight loss- and exercise-related amenorrhea. Kisspeptin is an endogenous neuropeptide, encoded by the *KiSS-1* gene and activates the G protein-coupled receptor (GPR54) – kisspeptin/GPR54 system. It is produced mainly in hypothalamus. The main role of kisspeptin is activation of hypothalamus-pituitary-ovarian axis (↑ GnRH release).

Inhibins are glycoproteins secreted by granulosa cells of early antral ovarian follicles.

The main inhibins function is inhibiting hypothalamic FSH production and inhibiting GnRH secretion during menstrual cycle (feedback mechanism).

MATERIAL

41 women with diagnosed FHA were enrolled to the study.

Criteria for FHA diagnosis were as follows:

- at least a 3 month history of amenorrhea not due to pregnancy,
- at least one documented less than 5 U/ml serum LH levels,

Control group - healthy women, normally menstruating with normal BMI.

METHODS

Medical history was conducted in patients with FHA and controls. Particular attention was paid to such aspects as duration of amenorrhea, weight loss in kilograms.

Serum kisspeptin, inhibin B, total inhibin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) levels were measured using ELISA methods.
Body mass index (BMI) was estimates in patients with FHA and controls.
RESULTS
Serum kisspeptin concentrations in patients with functional hypothalamic amenorrhea were statistically (p<0,0271) lower in comparison to control group.
Serum inhibin B concentrations in patients with functional hypothalamic amenorrhea were statistically (p<0,0001) lower in comparison to control group.
Serum total inhibin concentrations in patients with functional hypothalamic amenorrhea were statistically (p<0,0001) lower in comparison to control group.
CONCLUSIONS
Decreased serum kisspeptin concentrations in women with functional hypothalamic amenorrhea could be the cause of decreased activation of hypothalamus-pituitary-ovarian axis - GnRH release.
Low serum inhibin B and total inhibin concentrations in patients with functional hypothalamic amenorrhea could be the proof of inhibited ovarian function.

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Vitamin D Status in an Infertile Population. Roohi Jeelani, Ismail Mert, Valerie Shavell, Rashmi Bolinjkar, Elizabeth E Puscheck, Michael P Diamond. *Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*
Objective: Aside from bone metabolism, Vitamin D has been shown to play a key role in various health aspects including fertility. Our aim was to evaluate vitamin D levels in an infertile population, hypothesizing that there is a strong correlation between suboptimal vitamin D levels and infertility.
Design: Retrospective study.
Materials and Methods: Charts of women who presented for infertility evaluation from 2010 to present and whose vitamin D level was evaluated were reviewed. Vitamin D levels were stratified according to the following parameters: <10 ng/ml = deficiency, 11-30 ng/ml = insufficiency, 30-100 ng/ml = sufficient, and 40-65 ng/ml = optimal for health. Chi-square and t-tests were utilized for statistical analysis.
Results: Of the 110 women who were evaluated for infertility, vitamin D levels were assessed in 19 patients (17.3%). Of these patients, 15 (78.9%) had suboptimal levels of vitamin D (4 were deficient and 11 were insufficient). Only 2 of the 4 patients who had sufficient vitamin D levels were in the 'optimal for health' category. There were no significant differences with respect to age, gravidity, parity, or race between those who were suboptimal and those who were not (p>.05). The most common diagnosis in the suboptimal group was polycystic ovarian syndrome (40.0%), mean body mass index (BMI) was 25.4 ± 7.1 kg/m^2, and baseline estradiol was 96.2 ± 157.8 pg/ml, FSH 49.8 ± 113.6 mIU/ml, and antral follicle count 19.1 ± 10.8. These measures were not associated with vitamin D levels (p>.05). All patients who had a suboptimal vitamin D level were started on vitamin D supplementation. Of the 7 patients who achieved clinical pregnancy, 3 miscarried in the first trimester.
Conclusions: There is a high prevalence of suboptimal vitamin D levels in women undergoing evaluation for infertility. This strong association merits assessment of vitamin D levels in the infertile patient population. Further investigation needs to be conducted to determine what level is optimal for fertility.

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To Examine the Association between Suboptimal Vitamin D Status and Diabetes in a Pregnant Cohort in Ireland. Grace M Neville,[1] Robert O'Sullivan,[1] Yue Zhang,[2] Mairead Kiely,[2] Mairead N O'Riordan.[1] *[1]Anu Research Centre, University College Cork, Cork, Ireland; [2]School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.*
A high prevalence of suboptimal Vitamin D status in the Irish population has previously been reported. In international studies of Vitamin D status in pregnancy, controversy exists as to whether differences exist between those who have diabetes and those without.
The primary aim of this study was to assess the Vitamin D status of a cohort of pregnant women with diabetes attending Cork University Maternity Hospital, which lies at a latitude of 51°54'N.
Serum 25-hydroxy Vitamin D (25(OH)D) of 52 pregnant women with a diagnosis of diabetes was measured via ELISA assay in addition to analysis of other participant characteristics. These results were compared to a previously collected database of serum 25(OH)D levels of 264 non-diabetic pregnant women.Arbitrary definitions in line with current expert opinion and based primarily on studies into Vitamin D's function in calcium regulation and bone health were chosen as outlined.

Serum 25(OH)D Categories

Serum 25(OH)D nmol/L	Description
<25	Deficient
<50	Insufficient
<80	Suboptimal
>80	Optimal

A statistically significant difference between serum 25(OH)D levels in the 2 groups was not observed. However, just 7.2% of non-diabetics and 3.5% of diabetics achieved the serum 25(OH)D level considered optimal (>80nmol/L).

Baseline Characteristics of Diabetic and Non-Diabetic Cohorts

	Non Diabetic		Diabetic			
	Mean Value	n	Mean Value	n	p=0.05	Statistical Significance
25(OH)D nmol/l	43.09(±21.55)	264	39.64(±19.06)	52	0.284	No
Age (Years)	29.95 (±5.68)	264	32.73 (±5.32)	52	0.001	Yes
BMI	26.11 (±4.89)	184	30.00 (±6.69)	51	0.000	Yes
Gravida	2.25 (±1.42)	264	2.63 (±1.69)	52	0.088	No
Parity	0.89 (±1.11)	264	1.08 (±1.06)	52	0.274	No
Fetal Weight	3403.25(±574.94)	221	3439.62(±584.41)	52	0.683	No
Ethinity	1.9% Non Caucasian	264	17.3% Non Caucasian	52	0.000	Yes

n=number of participants on whom data was available
Suboptimal Vitamin D status was identified in both populations. No statistically significant difference between diabetics and non-diabetics was seen in our population which adds to uncertainty that currently exists in this area. Factors such as ethnicity and seasonal variance are likely to have a greater impact on Vitamin D levels then the diabetic status. Suboptimal Vitamin D levels are significantly reduced in both our populations and would suggest the need for surveillance and possibly treatment.

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Gray Matter Volume of the Emotional Cerebellum in Premenstrual Dysphoric Disorder. Andrea Rapkin,[1] Steven Berman,[2] Edythe London.[2] *[1]OBGYN, UCLA; [2]Psychiatry & Biobehavioral Sciences, UCLA.*
Premenstrual dysphoric disorder (PMDD) is a neuroendocrine disorder characterized by affective symptoms including irritability, depression, and anxiety during the luteal phase of the menstrual cycle. We reported that women with PMDD had greater relative glucose metabolism, an index of local brain function, than control women, in parts of the cerebellum linked to emotional processing. Because functional activity can increase brain structure, we quantified grey matter structure in the original research participants. We hypothesize that cumulative increases in activity of a distributed cerebellar network associated with emotional processing would result in higher cerebellar grey matter volume (GMV) in women with PMDD.
Women ages 20-41 years were screened using the Daily Rating of Severity of Problems and assigned to a PMDD (n=12) or control group (n=13). Grey matter structure was assessed in follicular and late luteal phases by high-resolution MRI. Cerebral GMV was assessed by voxel-based morphometry (VBM) through statistical analysis using SPM8 software, assessing differences between the PMDD and control group in GMV, and covariance between GMV and age. Findings were then characterized via region-of-interest (ROI) analyses.
Women with PMDD had higher GMV than controls in a cluster of 2,228 voxels in the cerebellum (whole-brain corrected p=0.035). In women under 30, the mean GMV was not significantly higher in the PMDD group than controls (mean [sd]= 72 [.06] vs .66 [.05] p=.12). In contrast, for women over 30, the mean GMV was significantly higher in the PMDD group (mean [sd]=.76 [.03] vs .62 [.04] p=.0002). Both effects covered a greater proportion of ROIs associated with emotional processing.
Women with PMDD had greater GMV than control women in the "limbic" cerebellum, but in no other brain area. The relationship of age to GMV showed a similarly localized group difference. The difference in brain structure occurred in locations where women with PMDD exhibited greater relative glucose metabolism than controls during the late luteal phase. Studies of the relationship between age-related loss in brain structure and cognition have also implicated the cerebellum. PMDD may protect against age-related loss of GMV in the "emotional" cerebellum. It remains to be determined if this is due to repeated symptom-related increases in activity and whether the effect has implications for treatment of PMDD and other disorders.

F-365

Ovarian Reserve Markers in Reproductive Age Women with Systemic Lupus Erythematosus. Olivio B Malheiro,[1] Carolina P Rezende,[2] Gilda A Ferreira,[1] Fernando M Reis.[2] *[1]Department of Locomotor System Medicine, University of Minas Gerais, Belo Horizonte, Brazil; [2]Department of Obstetrics and Gynecology, University of Minas Gerais, Belo Horizonte, Brazil.*

Objective: The aim of this study was to evaluate if there are differences in ovarian reserve markers in systemic lupus erithematosus (SLE) patients compared to controls, and to evaluate their relationship with clinical and treatment features of SLE patients.

Methods: This was a controlled cross-sectional study including 27 women with SLE and 27 controls. All participants were between 18 and 40 years, were eumenorrheic and did not use hormone therapy or hormone contraceptives in the past 6 months. Clinical data were assessed at regular follow up of disease, and patients provided blood samples to measure follicle stimulating hormone (FSH) and anti-mullerian hormone (AMH) concentrations, and underwent transvaginal ultrasound for antral follicle count. All procedures were made at early follicular phase of menstrual cycle. The study was approved by the IRB and all participants gave written informed consent.

Results: We found no difference between SLE group and control group at analysis of AFC [7 (5 – 13) vs. 11 (7 – 12), p=0.076], FSH [6.44 (4.19 – 7.69) vs. 7.5 (6.03 – 8.09) mUI/ml, p=0.135] and AMH levels [1.23 (0.24 – 4.63) ng/ml vs. 1.52 (1.33 – 1.88) ng/ml, p=0.684]. However, AMH values in SLE group were more heterogeneous compared to control group. The presence of nephritis and the cumulative dose of cyclophosphamide were factors individually related to reduced ovarian reserve, by association with lower values of AFC and AMH. At multivariate logistic regression, AMH was inversely related with corticosteroid doses in the follow-up (adjusted OR 0.95, 95%CI 0.894-1.000, p=0.50) whereas AFC was reduced in women with higher disease scores (adjusted OR: 0.14, 95% CI 0.025-0.841, p=0.031).

Conclusion: SLE patients who were eumenorrheic had average values of ovarian reserve markers similar to controls. However, AMH had a larger variation in SLE women, needing evaluation of other markers to clarify the best clinical application for it. Ovarian function is more compromised in patients with nephritis, higher cumulated dose of cyclophosphamide and with higher disease damage scores.

S-001

Somatic Development in Pre-Pubertal Mice Treated with Chyclophosphamide and Subsequent Estrogen Replacement. Laura Detti,[1] Daniel C Martin,[1] Robert W Williams,[2] Natalia Schlabritz-Loutsevich,[1] Rebecca A Uhlmann.[1] *[1]Obstetrics and Gynecology, University of Tennessee, Memphis, TN, USA; [2]Anatomy and Neurobiology, University of Tennessee, Memphis, TN, USA.*

Background: Disruption in the normal timing of female puberty, such as in pre-pubertal cancer treatments, can cause abnormal somatic development. We sought to evaluate the impact of different timing of estrogen therapy on the somatic development of pre-pubertal mice treated with therapeutic doses of cyclophosphamide.

Materials and Methods: Inbred C57BL/6J female mice were randomized to receive placebo (controls, n=6) or 120 mg/kg of cyclophosphamide on day 18 of life. The treated mice were then randomized to receive placebo (group 1, n=9) or estradiol pellet insertion on day 21 (group 2, n=10), day 45 (group 3, n=12), and day 67 of life (group 4, n=9). Pellets released 2 µg of 17 ß-estradiol valerate per day to obtain sustained serum levels of 50-100 pg/mL for 90 days. All mice were euthanized at day 95 of life. Body weight and length, uterine and ovarian weight and right femur length and weight were measured. Data were analyzed using ANOVA and t-test (SPSS v19).

Results: Body weight and length and BMI (kg/m2) did not differ among groups. The femur was shorter and weighed less in mice treated with cyclophosphamide (groups 1-4) than in controls (1.36 cm, 95% CI: 1.25-1.46 vs. 1.58 cm, 95% CI: 1.48-1.68, respectively; p<0.001). In addition, group 1 (no estradiol) femurs were the same length but weighed less than those from groups 2-4 (48 µg, 95% CI: 45-51, vs. 55 µg, 95% CI: 52-57, respectively; p=0.04). Uterine weight was lower in group 1 than in controls (46 µg, 95% CI: 43-49, vs. 62 µg, 95% CI: 59-65, respectively; p=0.006) and in groups 2-4 (98 µg, 95% CI: 54-180; p=0.02), but was not different in controls vs. groups 2-4. Ovarian weight did not differ among groups.

Conclusions: Cyclophosphamide treatment in pre-pubertal mice appears to negatively affect femur and uterine development, yet with no observable effect on adult body length and weight. Estrogen treatment restored normal uterine development by adulthood, regardless of the timing of administration. However, there was no similar recovery of femur length, and bone mass was only partially recovered.

S-002

Effect of Vitrification on Aurora Kinase A Expression and Germinal Vesicle Breakdown in Isolated Mouse Oocytes. Joseph Doyle, Ho Joon Lee, Kaisa Selesniemi, Sanaz Ghazal, Bo Rueda, Jonathan Tilly. *Vincent Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA.*

Objective: Vitrification of germinal vesicle (GV) oocytes negatively affects in vitro maturation (IVM). Aurora kinase A (AKA) is critical for meiotic spindle assembly during oocyte IVM. This study sought to determine if defects in oocyte IVM caused by vitrification are tied to changes in AKA expression.

Methods: GV oocytes were collected from young adult B6D2F1 female mice 40-44 hours after intraperitoneal injection of 7.5 IU of PMSG. Vitrification was performed immediately after collection. Viable GV oocytes with normal post-thaw morphology were matched with fresh (non-vitrified, control) GV oocytes. Both groups of oocytes were matured in human tubal fluid and data were collected at 0, 4, and 8 hours after culture initiation. Rate of GVBD was determined by light microscopy, whereas anti-tubulin staining of the spindle apparatus and DAPI staining of chromatin were assessed by fluorescence microscopy. In parallel, AKA mRNA and protein levels were assessed.

Results: Delayed GVBD was evident in vitrified versus control oocytes after 4 hours of IVM; these differences resolved by 8 hours. Normal spindle assembly showed a similar pattern of delay in vitrified versus control oocytes. AKA mRNA was detected in control and vitrified oocytes prior to culture, with higher levels present in the vitrified group. Contrasting this, Western blot showed that AKA protein was reduced in vitrified compared to control oocytes at 0 hours. These differences in AKA mRNA (higher) and protein (lower) were maintained through 4 hours of culture. By 8 hours, we no longer detected differences in AKA mRNA or protein between the two groups of oocytes.

Conclusions: IVM of vitrified GV oocytes is associated with delayed GVBD and spindle apparatus assembly in comparison to fresh GV oocytes matured in parallel. These events were accompanied by altered expression of AKA in vitrified oocytes, with the inverse relationship between AKA mRNA and protein indicative of a potential impairment in mRNA translation caused by vitrification. We conclude that vitrification has a negative impact on oocyte maturation machinery, leading to delayed GVBD and meiotic spindle organization during IVM.

S-003

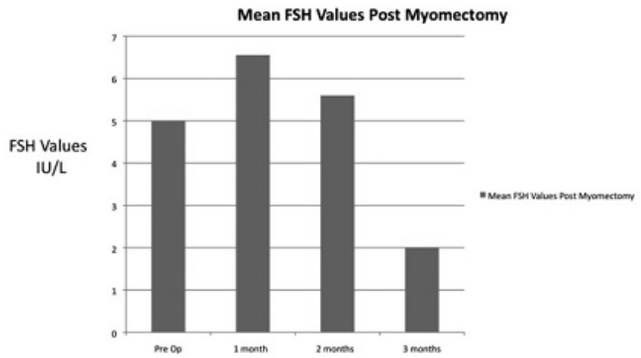
Ovarian Function Is Not Negatively Impacted by Myomectomy. Izabella Khachikyan, Gary Levy, Lynnette Nieman, Alicia Armstrong. *Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute for Child Health and Human Development, National Institute of Health, Bethesda, MD, USA.*

Objective: To evaluate the impact of abdominal myomectomy on ovarian function in reproductive age women.

Hypothesis: Myomectomy does not impact ovarian function.

Materials and Methods: Serum FSH levels were measured pre and post operatively in reproductive age women undergoing myomectomy in a randomized prospective clinical trial evaluating the use of a selective progesterone receptor modulator in the treatment of leiomyoma. Nine African American women underwent myomectomy. No patients were on the study drug during their biochemical evaluation.

Results: The patients ranged in age from 35-47 (mean age 39.4+/- 4). FSH values were obtained in the nine patients who underwent abdominal myomectomy. None of the patients evaluated demonstrated elevated pre operative FSH values. The mean FSH values pre operatively (5.00 IU/L), did not differ from values 1 month (6.56 IU/L), 2 months (5.6 IU/L) and 3 months (2.0 IU/L) post operatively.



Conclusion: It is widely assumed that myomectomy is the mode of therapy that has the least impact on ovarian reserve. Data to support this belief, and the practice of preferentially offering this treatment to women who desire fertility, is lacking. This small case series suggests that myomectomy does not impair future gonadal function. Larger studies with biomarkers such as antimullerian hormone are necessary to definitively evaluate the impact of myomectomy on ovarian reserve.

S-004

Withdrawn by Author

S-005

Reproductive Outcomes of a Cohort of Women Undergoing Conservative Progestin Therapy for Complex Atypical Hyperplasia or Grade 1 Endometrial Carcinoma. Rashmi Kudesia,[1] Tomer Singer,[2] Isaac Kligman,[2] Thomas C Caputo,[1] Divya Gupta,[1] Zev Rosenwaks.[2] [1]*Obstetrics & Gynecology, Weill Cornell Medical College, New York, NY, USA;* [2]*The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA.*

OBJECTIVE: Given US trends in reproduction and obesity, the number of women seeking assisted reproduction while having endometrial pathology is rising. Though women with complex atypical hyperplasia (CAH) or grade 1 carcinoma may seek to clear their pathology with oral and/or intrauterine progestins and then attempt pregnancy, data on success rates and safety is limited. In following a cohort of patients treated in our center, we evaluate our hypothesis that conservative therapy leads to a clinically significant pregnancy rate.

SETTING: Academic medical center.

METHODS: Pathology and billing codes were used to identify eligible patients who were less than 45 years of age with a pathologic diagnosis of CAH or grade 1 carcinoma and intent to seek fertility-sparing treatment. Demographic, pathologic and treatment data were obtained; reproductive and oncologic outcomes were analyzed.

RESULTS: Seventy-five possible patients were identified, of which 27 patients met criteria. Twenty-three sought or had already established fertility consultation, though only 14 ultimately underwent treatment. Of these, 2 received care outside our center and both ultimately had children via gestational surrogate. Twelve had IVF in our center. Eleven initiated their first cycle within 24 months of initial diagnosis; one patient waited 5 years. Five clinical intrauterine pregnancies (42%) and 4 live births (33%) resulted.

Statistic	Value
Mean age at diagnosis	38.1
Mean age at retrieval	40
Mean BMI	27.8
Distribution of diagnoses	CAH=12, carcinoma=15
Mean # of IVF cycles for pts with live birth	2.5
Mean peak endometrial stripe	9mm
Pregnancy rates	Clinical IUP=42%, Live Birth=33%
Patients ultimately with hysterectomy	10

CONCLUSIONS: Though live birth can be achieved in this patient population, the success rates are negatively impacted by persistence or progression of disease. Many questions remain to be addressed. Patients should be counseled carefully regarding their overall likelihood of live birth and factors that may impede their success.

S-006

ART Outcomes May Differ by Cancer Diagnosis. Mary Ellen Pavone,[1] Jennifer E Hirshfeld-Cytron,[2] Angela Lawson,[1] Kristin Smith,[1] Susan Klock.[1] [1]*Obstetrics and Gynecology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA;* [2]*Obstetrics and Gynecology, University of Illinois Medical Center at Chicago, Chicago, IL, USA.*

Objective: We have previously shown that there are minimal differences in IVF outcomes between cancer patients and age matched infertile controls. Here, we explore if differences exist based on cancer diagnosis.

Materials and Methods: Retrospective analysis of 63 patients diagnosed with cancer that opted for fertility preservation (FP) in the form of embryo or oocyte cryopreservation.

Results: A total of 315 patients expressed initial interest in FP. Of those, 133 patients requested consultation for either embryo or oocyte cryopreservation. Of the 63 patients reviewed who underwent a successful oocyte harvest, 34 had breast cancer, 18 had hematologic malignancies and 11 had other cancer diagnoses (colon, brain and ovarian cancer). All patients were stimulated using an antagonist protocol. Comparing the three groups, we found that breast cancer patients were statistically older (33yo breast vs 28.6yo heme vs 30.3yo other) and were less likely to have received prior chemotherapy (3% vs 17% vs 27% of subjects). We found no differences in the amount of gonadotropins used, number of days stimulated, peak E2 and number of mature oocytes on ultrasound. We found a statistically higher number of oocytes retrieved in patients with breast cancer compared to hematologic and other (15 vs 10.1 vs 6.4 $p<0.05$), statistically higher number of mature oocytes in breast compared to hematologic and other (11.1 vs 7.1 vs 6.4, $p<0.05$) and more zygotes created in breast vs heme (avg=7.7vs 4.1, $p<0.05$).

Conclusions: Discussing FP is an important aspect of cancer care in reproductive aged women. Despite being older, breast cancer patients tended to have a better response to ovarian stimulation. This may be partly explained because these women were less likely to have undergone prior chemotherapy. With proper screening and counseling, many patients will be appropriate candidates for FP. We believe a difference in ovarian response due to cancer diagnosis is vital information to disseminate to other programs offering fertility preservation.

S-007

Mesial Incision for Laparoscopic Dermoid Cystectomy: A New Ovarian Tissue-Sparing Technique. Rita Mocciaro, Roberta Venturella, Angela Sacchinelli, Michele Morelli, Zullo Fulvio. *Obstetrics and Gynecology, University "Magna Graecia".*

Background: Dermoid cysts represent 21% of all ovarian tumors and are the most common germ cells tumor, afflicting principally woman in reproductive age.

The standard procedure to enucleate dermoid cysts provides a laparoscopic antimesial incision. However, considering the greater ovarian cortical thickness at mesial side, we hypothesized that incision in this area could provide a better identification of cleavage plane reducing ovarian wall electrocauterization, so sparing ovarian residual function.

Design: Randomized controlled trial.

Patients: 66 women, 18 to 45 years of age, with homogeneous basal FSH levels, affected by unilateral (61 patients) or bilateral (5 patients) dermoid cysts, divided into study group (33 patients) and control group (33 patients).

Interventions: Laparoscopic dermoid cystectomy by mesial incision (study group) or antimesial incision (control group).

Main outcomes: Post-operative ovarian reserve (variation of FSH levels, basal antral follicle number, mean ovarian diameter, peak systolic velocity at 3 and 12 months after surgery). Operative times, spillage of intracyst contents rate, chemical peritonitis rate, intraoperative blood loss (Δ Hb), were also evaluated as secondary outcomes.

Results: Median FSH values were significantly lower in the study group than in the control group, at 3 and 12 month follow-up. Median basal antral follicle number, median ovarian diameter and median peak systolic velocity were significantly higher in the study group than in the control group, at 3 and 12 month follow-up. A significant difference was found between study and control group in term of operative time (48 vs. 76 minutes, respectively; $P < 0.001$) and spillage of intracyst content rate (3% vs. 21% $P < 0.05$). None developed chemical peritonitis. Δ Hb was higher in the study group than in the control group (1 vs. 0.8 g/dl), but this difference was not statistically significant.

Conclusions: The mesial incision appears to be a tissue-sparing technique, not affecting ovarian reserve.

S-008

Intrauterine Evolution of Fetal Cardiac Defects Diagnosed between 11 and 14 Weeks of Gestation. Maria Chiara Autuori, Maria Bellotti, Giulia Rognoni, Serena Migliaccio, Elisa Matarazzo, Camilla Bulfoni, Arianna Prada, Anna Maria Marconi. *Obstetrics and Gynecology, DMSD San Paolo Medical School - University of Milano, Milano, Italy.*

Objective: To evaluate the in utero evolution of cardiac anomalies [CHD] identified <14 weeks of gestation.

Methods: We retrospectively analyzed the evolution of cardiac and extra-cardiac morphology in fetuses with a diagnosis of CHD performed by US in the first trimester.

Results: 13 fetuses met the inclusion criteria. The gestational age at the first anomalous scan and at definitive diagnosis were 12.3 ± 1 and 18.5 ± 7 wks. Fetal karyotype was normal in 7 cases, not investigated in 3 and abnormal in 3 cases (1trisomy 21, 1 trisomy 6 mosaicism and 1 monosomy 13 mosaicism) . Extra-cardiac anomalies were present in 8 cases. 10 fetuses had increased NT. A definitive cardiac diagnosis was obtained at the first trimester scan in 4 cases: 1 pulmonary artery hypoplasia at 12 wks had a definitive diagnosis of Tetralogy of Fallot at 15 wks; 1 atrioventricular septal defect (AVSD) at 12+1 wks with unbalanced ventricles; 1 truncus arteriosus plus AVSD at 13+6 wks and 1 ventricular septal defect (VSD) with right-left shunt at 13+1 wks. An evolution of the first trimester cardiac feature was observed in 8 cases:

- 4 with right ventricular dominance: 1 case with right ventricle to left ventricle ratio (RV/LV) = 1.2 at 13+4 wks had proportionate ventricles at 16 wks whereas 3 cases with RV/LV ≥ 1.7 became hypoplastic left ventricle (1 isolated and 2 associated with double outlet right ventricle at 17, 12+3 and 15+2 wks respectively)
- 1 left ventricular dominance plus multiple muscular VSD at 12+4 evolved into mild disproportion associated with severe myocardial hypertrophy and persistence of VSD at 18+5wks
- 1 right ventricle and pulmonary artery's hypoplasia with tricuspid stenosis and VSD with left to right shunt at 13+6 wks evolved into mild disproportion of ventricles with tricuspid stenosis at 34+2 wks
- 1 isolated VSD and 1 VSD associated with myocardial hyperechogenicity resolved within 21 wks
- 1 tricuspid regurgitation at 12+6 and 15 wks had normal heart at 20 wks but Aortic Coarctation was diagnosed at 32 wks.

Conclusions: Hypoplasia of the great arteries or ventricles could be either a definitive feature of the fetal heart in the first trimester or an evolution of early cardiac ventricular disproportion. In presence of anomalous or doubtful first trimester cardiac scan, longitudinal evaluation is mandatory.

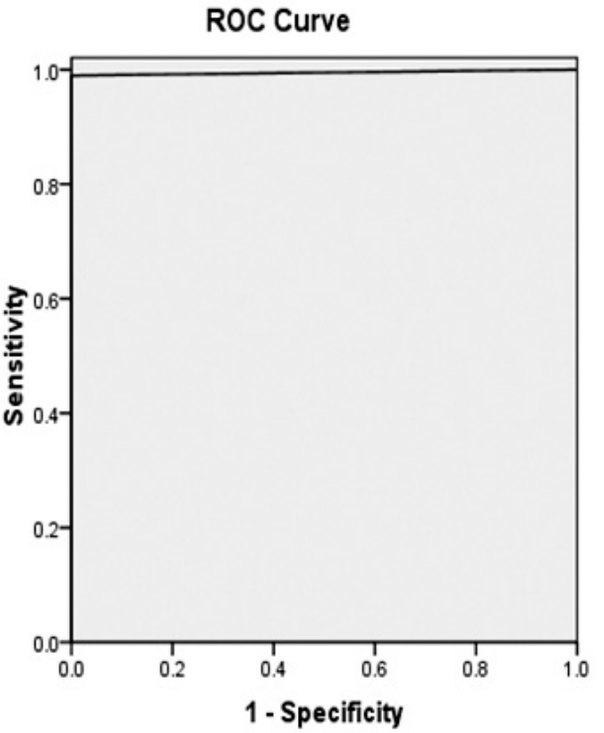
S-009

Antenatal Diagnosis of β Thalassemia: Comparison of DNA Analysis and Hb Typing. Piya Chaemsaithong,[1] Panyu Panburana,[1] Prakuywan Kadekasem,[2] Ampaiwan Chuansamrit,[2] Boonsri Chanrachakul,[1] Patama Promsonthi.[1] *[1]Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*

Objective: To propose the simple, reliability method and cut-off value of HbA level from cordocentesis by either high performance liquid chromatography (HPLC) or automated capillary electrophoresis (CE) for the prenatal diagnosis of beta thalassemia.

Method: Cordocentesis results from the couple at risk for beta thalassemia major and intermedia during 2000-2010 were reviewed. Cordocentesis was performed during 18-22 weeks of gestation. Fetal blood was analyzed using HPLC or CE. Identification of β mutations were performed using the allele-specific PCR for 10 common mutations.

Results: Two hundred nineteen fetal blood specimens at 18-22 weeks were recruited. Of those, 164 cases were analyzed by DNA analysis. β-globin gene mutations were identified in 134 cases. Among these, βthal/Hb E, heterozygous Hb E, heterozygous β thalassemia, normal and homozygous β thalassemic fetuses were diagnosed in 35 (26.12%), 35 (26.12%), 33 (24.63%), 28 (20.90%) and 3 (2.24%), respectively. Mean levels of Hb A were 0.97 ± 0.84% (range 0-1.5%) in homozygous β thalassemia and 0.1 ± 0.27(range 0-1.2%) in βthal/Hb E. The mean levels of Hb A were 3.93 ± 2.31 (range 1.7-11.9%) in β-thalassemia heterozygote, 3.52 ± 2.07 (range 1.0-10.5%) in Hb E heterozygote and 5.81 ± 7.68 (range 3.1-12.5%) in normal fetuses. The levels of Hb E (A_2) in β thal/Hb E and heterozygous Hb E were 1.29 ± 0.24 and 1.50 ± 1.36. The cut-off value of Hb A at 1.6 provided the 100% sensitivity, 99% specificity, diagnostic accuracy of 99.3% ($p<0.01$) and AUC 0.995.



Conclusion: The absence or low level of Hb A by in cord blood is the good indicator to identify the affected fetuses. This method is simple, quick and reliable. It can be used as an alternative method for prenatal diagnosis of beta thalassemia in the hospital that cannot perform DNA analysis.

S-010

Assessment of Fetal Growth Restriction in Pregnancies Complicated by Low Pregnancy Associated Plasma Protein-A on Routine First Trimester Aneuploidy Screening. Christine A Laky,[1] Mark J Wehrum,[1] Genevieve R Cozzini,[1] Herb Malkus,[2] Joshua A Copel.[1] *[1]Maternal-Fetal Medicine, Yale University School of Medicine, New Haven, CT, USA; [2]Laboratory Medicine, Yale-New Haven Hospital, New Haven, CT, USA.*

OBJECTIVE: Low pregnancy associated plasma protein has been associated with fetal growth restriction and low birth weight. The objective of this study was to further define this association at our institution.

STUDY DESIGN: We performed a retrospective case control study of 16503 patients who received first trimester screening at Yale-New Haven Hospital and Yale University School of Medicine and who delivered between May 10, 2004 and July 3, 2011. There were 1002 patients with singleton pregnancies with a low PAPP-A of <0.40 MoM and 618 of these patients had PAPP-A < 0.34 MoM. We reviewed prenatal records including ultrasound data and delivery records. Complete data was available for 509 patients.

RESULTS: Of the patients with low PAPP-A on first trimester screening, 15.9% had low-birth weight babies, defined as birth weights < 10%. This prevalence of low birth-weight babies in the low PAPP-A group was statistically significant. Patients with a low PAPP-A were also significantly older at delivery with a mean age of 33.4 yrs compared to 31.6 yrs for the control group. There was a higher proportion of males born to the low PAPP-A patients, 56.6% males to 43.3% females, compared to the expected ratio of 51.2% males to 48.8% females. There was no significant difference in mode of delivery with 38.8% cesarean delivery rate which is consistent with recently published data for mode of delivery at YNHH.

CONCLUSION: This study shows an increased risk of low-birth weight infants in patients with low PAPP-A on first trimester screening compared to the general population. This is consistent with previously published data and reiterates the need for growth ultrasound in the third trimester to identify those fetuses at risk for potential complications and need for possible intervention.

S-011

Antenatal Evaluation of Sonographically Absent/Small Fetal Stomachs. Katherine A Latimer, Jessica Bienstock, Nancy A Hueppchen. *Gynecology & Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MS, USA.*
Objective: Evaluate neonatal outcomes of fetuses with sonographically small or absent fetal stomachs and propose an evidence-based management algorithm.
Methods: A retrospective observational study was performed including fetuses diagnosed with sonographically small/absent stomachs at Johns Hopkins Hospital from 5/95-12/10. Antepartum and neonatal records were reviewed. Terminations and fetuses from monochorionic, multiple gestations were excluded from neonatal outcomes analysis. Cases were sorted by amniotic fluid (AF) status and presence/absence of sonographic anomalies, with a focus on *abnormal outcomes* not predicted by initial sonogram—including unexpected anomalies, fetal demise, in-utero infections, feeding issues in infants born after 35 weeks, GERD, and aneuploidy.
Results: The cohort contained 584 cases (n=93 absent, 491 small). Aneuploidy incidence varied between normal AF volume cases (3%) and cases with polyhydramnios (14%, $p<0.004$) or oligohydramnios (6%, $p<0.21$). Total abnormal neonatal outcomes, including those diagnosed antenatally by sonogram, occurred in 72% of absent stomach cases and 45% of small stomach cases ($p<0.0001$). Unanticipated, abnormal outcomes occurred in 47% of absent stomach and 36% of small stomach cases ($p<0.15$). The overall incidence of unpredicted GI abnormalities including imperforate anus, GERD, and feeding issues was 2%, 4%, and 9%, respectively. Esophageal atresia (EA) was noted in 14% of polyhydramnios cases. Unanticipated cardiac anomalies were common across groups (5%). With normal AF and isolated absent/small stomach, the incidence of unpredicted, abnormal outcomes was similar regardless of antenatal resolution or persistence (16% vs 15%, $p= 1.0$) of the abnormal stomach.
Discussion: Identification of a small or absent fetal stomach should prompt evaluation for additional abnormalities with attention to fluid status as a potential risk modifier. Offer karyotype whenever polyhydramnios or additional sonographic abnormalities are noted. Consider fetal echocardiogram due to higher incidence of congenital heart disease in these pregnancies compared to the general population. Patients should be counseled about potential for feeding difficulties and unidentified GI abnormalities. Consider MRI to evaluate for EA when polyhydramnios is present. Resolution or persistence of an isolated stomach abnormality did not alter the incidence of unanticipated, abnormal neonatal outcomes.

S-012

***prkca* Gene Deletion Prevents Maternal Diabetes-Induced Neural Tube Defects through Suppression of the Pro-Apoptotic JNK1/2 Signaling.** Xuezheng Li,[1] Cheng Xu,[1] E Albert Reece,[1,2] Peixin Yang.[1,2] *[1]Obstetrics, Gynecology & Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD, USA; [2]Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, USA.*
Activation of the Protein Kinase C (PKC) pathway is critically involved in the induction of diabetic embryopathy. Our previous studies have demonstrated that PKCa (*prkca*) is activated in diabetic embryopathy, and pharmacological inhibitors of PKCa significantly reduce hyperglycemia-induced Neural Tube Defects (NTDs). We have also demonstrated that the pro-apoptotic c-Jun-N-terminal kinase 1/2 (JNK1/2) mediates the teratogenicity of maternal diabetes. The present study was designed to determine the role of *prkca* gene in NTDs formation induced by maternal diabetes, and define the relationship between PKCa and JNK1/2 activation in diabetic embryopathy.
Methods: Embryonic day 11.5 (E11.5) and E8.75 embryos from non-diabetic Wildtype (WT) control (NC), WT diabetic mellitus (DM), non-diabetic PKCa knockout (ND-PKCaKO) and diabetic PKCaKO (DM-PKCaKO) mice in C57BL/6J background were used to determine NTDs incidences, the activation (phosphorylation) of JNK1/2 and its downstream effectors, and caspase 8 cleavage.
Results: *prkca* gene deletion did not affect glucose levels in diabetic mice because glucose levels in the DM-PKCaKO group were similar to those in the DM group but were two times more than those in the NC group and the ND-PKCaKO group. The DM-PKCaKO group had a 5.3 ± 2.3% NTDs rate which was similar to those of the ND group (0%) and the ND-PKCaKO (0%), but was significant lower than that of the DM group (30.9 ± 4.9%). Phosphorylated (p-) levels of JNK1/2, and its downstream effectors (c-Jun, ATF-2, Elk1 and FoxO3a) were assessed in the NC, DM and DM-PKCaKO groups. Levels of p-JNK1/2 p-c-Jun, p-ATF-2 and p-Elk1 in the DM group were significantly increased than those in the NC and the DM-PKCaKO groups. p-FoxO3a (inactivation) levels were significantly higher in the NC and the DM-PKCaKO groups than that of the DM group. Cleaved caspase 8 was robustly present in the DM group, but was absent in the NC and the DM-PKCaKO groups.
Conclusions: We conclude that targeted deletion of the *prkca* gene prevents maternal diabetes-induced NTDs via blockade of the pro-apoptotic JNK1/2-caspase 8 pathway. Our results support the causative role of PKCa activation in the induction of diabetic embryopathy.

S-013

Maternal Dry Blood Spot for Non-Invasive Fetal RhD Genotyping at First Trimester. Yali Xiong,[1] Indhu M Prabhakaran,[1] Eliezer J Holtzman,[3] Stacey Jeronis,[2] Dan A Liebermann,[1] Barbara Hoffman,[1] Ossie Geifman-Holtzman.[4] *[1]Fels Institute, Temple University School of Medicine, Philadelphia, PA, USA; [2]OBGYN, Temple University School of Medicine, Philadelphia, PA, USA; [3]Nephrology and Hypertension Institute, Sheba Medical Center, Tel-Aviv University, Tel Hashomer, Israel; [4]OBGYN, Drexel University, Philadelphia, PA, USA.*
Objective: To evaluate the use of maternal dry blood on Guthrie card obtained at the first trimester screen as a common source for first trimester screen, fetal gender determination and Fetal RhD genotyping.
Study Design: Guthrie card blood samples and peripheral blood samples were collected from RhD-negative pregnant women in the first trimester. Guthrie cards blood samples were pretreated in 10XTE buffer. Mononuclear layer was isolated from peripheral blood with Ficoll. DNA from both Guthrie cards and peripheral blood mononuclear layer was isolated using Qiagen mini blood kit. Primers and probes specific to fetal gender determination (male DSY14) gene and polymorphism sites (female) were used respectively. Probe specific Realtime PCR was processed including examining the Exon 4, 5, and 6 of RhD gene. Maternal and paternal cheek swabs were used for parental genotyping and follow up confirmation on the babies were obtained after delivery.
Results: Male Fetal DNA was confirmed to be present in the amplified samples using DSY14 gene. Samples that were not amplified for DSY14 and were subjected to polymorphic sites amplification (SO6) confirmed the presence of fetal DNA and correctly identified female fetus. The subsequent determination of fetal RhD type using the fetal DNA from the maternal dry blood spot was confirmed using DNA obtained from maternal blood in the second trimester and /or after delivery as well as by standard serology of the baby's blood. Fetus RhD genotype results was consistent between DNA obtained from Guthrie Card and from the mononuclear layer isolated from peripheral maternal blood. One alloimmunized patient had blood placed on Guthrie card as early as 5 weeks gestation and was incorrectly diagnosed.
Conclusions: Our study revealed that fetal DNA from maternal blood samples placed on Guthrie card during first trimester screen could also be used for fetal RhD genotyping and fetal sex determination. The availability of this early testing is important clinically not only for diagnosis of X-linked diseases but also for determination of fetal RhD type in RhD-negative pregnant patients.

S-014

Increased Inhibin A in IUGR Fetuses Is Associated with Extreme Abnormalities in Umbilical Artery Doppler. Amanda Roman,[1] Neeraj Desai,[1] David Krantz,[2] Jonathan Rosner,[1] Meir Greenberg,[1] Nidhi Vohra,[1] Burton Rochelson.[1] *[1]Maternal-Fetal Medicine, Hofstra North Shore-LIJ Medical School, NY; [2]Genetic Screening, NTD Labs/PerkinElmer, NY.*
OBJECTIVE: To determine whether second trimester serum biochemical analytes are useful in differentiating IUGR fetuses with abnormal umbilical artery (UA)Doppler versus those with normal UA Doppler.
METHODS: Retrospective study of singleton pregnancies diagnosed with IUGR (EFW <10th%ile) who underwent second trimester screening from 2002 to 2011. Corrected values of maternal serum analytes (alphafetoprotein (AFP), free-ßhCG, unconjugated estriol, and Inhibin A), UA Doppler, and delivery outcome data were collected. Cases with abnormal karyotype or congenital malformations were excluded. We defined abnormal UA Doppler as systolic/diastolic (S/D) ratio >95th%ile. Extreme abnormalities included those with absent or reversed end diastolic velocities (AREDV). Data were analyzed using ANOVA and Fischer's exact test. Serum analyte >97th%ile was considered increased.
RESULTS: We identified 210 IUGR fetuses. The mean of AFP, free-ßhCG, estriol and Inhibin A levels were significantly elevated in the IUGR/AEDV fetuses when compared to those with IUGR/normal Doppler (Table 1). Only increased Inhibin A was associated with IUGR/AREDV compared with IUGR/normal UA: 46% vs. 15% OR 4.7 (95% CI:1.8-12, P<0.05). Increased AFP and Inhibin A were more frequent in IUGR fetuses diagnosed ≤24 weeks than

those diagnosed > 24 weeks; for Increased AFP: 31% vs. 6.2% OR 5.1 (95% CI:2.0-13.1, P<0.001), and increased Inhibin A: 67% vs. 21% OR 9.2 (95% CI:2.9-28.4, P<0.0001).

CONCLUSION: Inhibin A >97th%ile may predict extreme abnormalities in Doppler for the IUGR fetus especially those diagnosed before 24 weeks. With this knowledge, antenatal surveillance could be begun early for those pregnancies at highest risk.

Table #1

Variables	IUGR AREDV N=59	IUGR Elevated SD ratio > 95th%ile N=20	IUGR Normal Doppler N=131	P value ANOVA
AFP MoM (Mean ± SEM)	1.64 ± 0.2	1.4 ± 0.2	1.24 ± 0.9	0.005
Free BhCG MoM (Mean ± SEM)	2.07 ± 0.2	1.37 ± 0.2	1.36 ± 0.11	0.01
uEstriol MoM (Mean ± SEM)	1.78 ± 0.2	0.95 ± 0.08	1.3 ± 0.2	0.007
Inhibin A MoM (Mean ± SEM)	2.9 ± 0.3	1.85 ± 0.73	1.68 ± 0.22	0.002
GA at diagnosis, wks (Mean ± SD)	25.8 ± 5.4	34.4 ± 3.1	34.9 ± 3.1	<0.0001
*P=<0.05 was considered significant				

S-015

Low PAPP-A in IUGR Fetuses Is Associated with Extreme Abnormalities in Umbilical Artery Doppler. Amanda Roman,[1] Neeraj Desai,[1] David Krantz,[2] Jonathan Rosner,[1] Monica Sood,[1] Meir Greenberg,[1] Nidhi Vohra,[1] Burton Rochelson.[1] *[1]Maternal-Fetal Medicine, Hofstra North Shore-LIJ Medical School, NY; [2]Genetic Screening, NTD Labs/PerkinElmer, NY.*

OBJECTIVE: To determine whether first trimester serum biochemical analytes are useful in differentiating IUGR fetuses with abnormal umbilical artery (UA) Doppler versus those with normal UA Doppler.

METHODS: Retrospective study of singleton pregnancies diagnosed with IUGR (EFW <10th%ile) from 2002 to 2011. First trimester screening results, UA Doppler, and delivery outcome data were collected. Singleton pregnancies with birth weight 10-90th%ile (AGA) served as controls. Cases with abnormal karyotype or congenital malformations were excluded. We defined abnormal UA Doppler as systolic/diastolic (S/D) ratio >95th%ile. Extreme Doppler abnormalities were absent or reversed end diastolic velocities (AREDV). Data were analyzed using ANOVA and Fischer's exact test.

RESULTS: We identified 184 fetuses with IUGR. PAPP-A and free βhCG means were significantly different in IUGR/AREDV. PAPP-A <3rd%ile was more frequent in fetuses with IUGR/AREDV (24%) and IUGR/elevated UA (8%) than those with IUGR/normal UA (4.3%) P<0.05 or with AGA controls(2.5%) P< 0.0001 (Table 1). PAPP- A <3rd%ile in IUGR/normal UA fetuses were not significantly different from the AGA controls (Table 2). Low PAPP-A was also more frequent in IUGR fetuses diagnosed ≤24 weeks than those diagnosed >24 weeks: 27% vs. 8.6% (OR 3.9 95% CI: 1.3-11.7 P< 0.05). Free βhCG >97th%ile did not have a significant association with any outcome.

CONCLUSION: PAPP-A < 3rd%ile may help to identify those fetuses at risk of developing early onset IUGR ≤24 weeks and IUGR associated with extremely abnormal UA Doppler (AREDV) and elevated UA S/D ratio. Free βhCG did not have the same association.

Table #1

Variable	IUGR AREDV	IUGR Elevated S/D Ratio >95th%	IUGR Normal UA Doppler	AGA	P Value* ANOVA
	N=50	N=20	N=114	N=3100	
PAPP-A MoM (Mean ± SEM)	0.67±0.1	0.69±0.09	1.03±0.07	1.09±0.01	<0.0001
F βhCG MoM (Mean ± SEM)	1.3±0.1	1.07±0.1	1.0±0.01	1.0±0.01	0.006
PAPP-A <3rd%ile, N(%)	12 (24)	4 (8)	5 (4.3)	78 (2.5)	<0.05
IUGR, GA wks at diagnosis (Mean ± SD)	26.3± 5.2	35.3±2.2	34.9± 3.8	NA	< 0.0001
*P= < 0.05 was considered significant					

Table #2 PAPP-A <3rd percentile

Variable	OR (95% CI)	P value*
IUGR/AREDV vs. IUGR/Normal UA Doppler	6.8(2.2-20	0.0004
IUGR/AREDV vs. AGA	12.2 (6.1-24)	<0.0001
IUGR/Elevated S/D Doppler vs. IUGR/Normal UA Doppler	5.4(1.3-22)	0.03
IUGR/Elevated S/D Doppler vs. AGA	9.6(3.1-29)	<0.0001
IUGR/AREDV vs. IUGR/Elevated S/D Doppler	1.2(0.3-4.5)	NS
IUGR/Normal UA Doppler vs. AGA	1.7(0.7-4.4)	NS
*P= < 0.05 was considered significant		

S-016

Evolution of Fetal Reductions to Singletons. Mara Rosner,[1] Stephanie Andriole,[2] David W Britt,[3] Juliana Gebb,[1] Peer Dar,[1] Mark I Evans.[2] *[1]Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine, New York, NY, USA; [2]Obstetrics and Gynecology, Mount Sinai School of Medicine, New York, NY, USA; [3]Department of Health and Sport Science, University of Louisville, Louisville, KY, USA.*

OBJECTIVE: Nearly 25 years ago, we (MIE) published the first US series of fetal reduction cases and concluded that reduction from triplets+ to twins improved perinatal outcome. We decided with our bioethicist that we would not reduce to singletons as 1. Every obstetrician could manage twins 2. Twin outcomes were "reasonable" 3. Twins would assist patients to reach their family planning goals and 4. Concern that more intervention would increase loss rates. As data emerged demonstrating the safety of CVS and reduction to singleton, we liberalized our approach.

METHODS: We retrospectively analyzed FR cases from 4 time periods in the past 25 years: 1988-1993 & 1999-2002 versus 2007-2008 & 2009-3/2011.

RESULTS: There has been a steady increase in the % of patients reducing to a singleton (p<0.001), who are older than those reducing to twins (p<0.02), and decreasing % of quads+ (p<.001) for whom reduction to singleton has higher loss rates.

Table 1. Triplet and higher order gestations undergoing FR

	% Quads+[2]	3→2	Age	% CVS[3]	3→1	Age	% CVS[3]
88-93[1]	58.3%	149/175 (85.1%)	38	0	26/175 (14.9%)	42	0
99-02[1]	47%	194/219 (88.6%)	37.5	38%	25/219 (11.4%)	41.9	80%
07-08	16.3%	82/106 (77.4%)	35.1	80.5%	24/106 (22.6%)	37	91.7%
09-3/11	14.6%	47/62 (75.8%)	34.5	87.2%	15/62 (24.2%)	37.5	86.7%

[1] historical data, previous pubs, [2] % of total patients with quads+, includes anomalies and mono/di [3] % CVS & FISH before FR

Table 2. Twins undergoing FR

	2→1	% of total cases[2]	Age	% CVS
88-93[1]	18	10.8%	37.9	0
99-02[1]	52	23.7%	38.2	71%
07-08	49	30.4%[3]	37.2	85.7%
09-3/11	56	39.6%[3]	38	91.1%

[1] historical data, previous pubs [2] twins/total number of cases [3] if exclude anomalies, 07-08 = 26.5% and 09-3/11 = 40.4%

CONCLUSION: 1. Increased risks of twins 2. More patients with prior children 3. Decreased higher order multiples and 4. Patient preference have increased the proportion of patients reducing to 1. This increase persists even when excluding anomalies and mono/di twins. Average age of all patients has decreased, but the age difference between those reducing to twins vs. singletons remains. Utilization of CVS when reducing to a singleton was adopted earlier than for twins. As fewer patients start with 4+, reduction to 1 is becoming main stream.

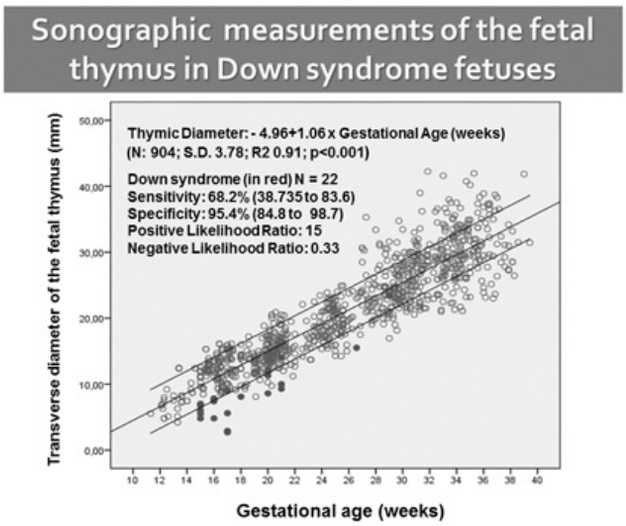
S-017

Clinical Diagnostic Performance of the Sonographic Fetal Thymic Measurements in Down Syndrome Pregnancies. Francisco Gamez,[2] Joaquin Santolaya-Forgas,[1] Juan DeLeon-Luis,[2] Jacobo Leopoldo Santolaya,[1] Luis Ortiz-Quintana.[2] *[1]Center for OBGYN Research and Mentorship, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA; [2]Obstetrics and Gynecology, Gregorio Maranon General Hospital, Madrid, Spain.*

This was a prospective and comprehensive US-study that included the measurements of the fetal thymus in 904 Control patients with well-dated pregnancies and 22 patients carrying Down syndrome fetuses confirmed by

antenatal testing. We used a GE-Logic US-equipment to measure the fetal Thymus at the level of the 3-vessel view of the heart as previously described. Sensitivity, Specificity and Likelihood ratios for detection of Down syndrome based on Thymic measurements were calculated. Figure 1 summarizes graphically and numerically our results.

Conclusion: sonographic evidence of fetal thymic hypoplasia can be used for Down syndrome screening purposes and to identify those at increased risk for T-cell disfunction and neonatal/childhood immunodeficiency.



S-018

A Comparative Study of 2-Dimensional vs. 3-Dimensional Sonographic Estimation of Fetal Weight. Ido Solt,[1] Nir Haya,[2] Udi Ergaz,[1] Khatib Nizar,[1] Israel Goldstein,[1] Yael Goldberg,[2] Zeev Weiner.[1] *[1]Obstetrics & Gynecology, Rambam Health Care Campus, Haifa, Israel; [2]Obstetrics & Gynecology, Carmel Medical Center, Haifa, Israel.*

Background: Many of the existing prenatal fetal weight estimation formulas were established over two decades ago. Since then and with recent advances in ultrasound technology, they have become increasingly valuable as an obstetric decision making tool, directly affecting labor management.

Although the fetal body is a voluminous mass, its weight is typically calculated by only two or three parameters, with a 10-15% deviation.

We hypothesized that using 3-dimensional (3D) ultasound, a new fetal weight estimation formula could be established based on head and abdomen volume, that would increase the accuracy of the sonographic estimation as compared to the actual fetal weight, and reduce the conventional 10-15% deviation.

Methods: The study population included 80 women presenting at our Prenatal Ultrasound Unit for third-trimester fetal evaluation. Fetal measurements were obtained, including bi parietal diameter (BPD), abdominal circumference (AC), femur length (FL), along with information on fetal gender, gestational age, history and complications, medical status of the patients, obstetric history and previous birth outcomes. Head and abdominal volumes were also obtained using the VOCAL™ software. Initial sections used for the VOCAL™ calculations were the conventional head and abdomen circumeference measurements.

Results: Women's average age was 30.5 (ranging 20-45) and gestational age averaged 39.3 weeks (ranging 36-42). High correlations were found between abdominal volume and AC (r=0.87), head volume and HC (r=0.82), and head volume and BPD (r=0.80).

Correlation coefficients with estimated fetal weight

Parameter	Mean	Standard Deviation	Correlation coefficient with EFW
Bi Parietal Diameter	9.3cm	0.4cm	0.615
Head Circumference	33.5cm	1.2cm	0.694
Head Volume	602cm³	82cm³	0.48
Abdominal Circumference	34.4cm	2.2cm	0.957
Abdominal Volume	942cm³	220cm³	0.715
Estimated Fetal Weight	3455gr	502gr	-
Birth Weight	3468gr	547gr	0.793

Preliminary curves of fetal abdomen and head volumes throughout the third term will be presented.

Conclusion: Significant correlations between the 3D volumes of the head and abdomen, and the two-dimensional HC, BPD, AC and estimated fetal weight (EFW) measurements, were demonstrated. Our preliminary results suggests that 3D volume measurements can be employed for more accurate assessment of fetal weight and size.

S-019

The Nuchal Translucency Examination, a Window to Early Diagnosis of Structural Fetal Anomalies. Ido Solt, Israel Goldstein, Boris Weizman, Khatib Nizar, Zeev Weiner. *Obstetrics & Gynecology, Rambam Health Care Campus, Haifa, Israel.*

Objective

To summarize six years experience of diagnosing structural fetal anomalies during an extended nuchal translucency (NT) examination.

Methods

The study population included all women who had a routine NT examination in our ultrasound division during the last 6 years. The sonographers were instructed to perform an extended NT examination by paying attention to fetal anomalies. Each examination was initially attempted transabdominally. Failure to obtain adequate views transabdominally was an indication for a transvaginal examination. When a structural fetal anomaly was detected or suspected, a full fetal anomaly scan was performed. When diagnosis could not be established, fetal anatomy scan was repeated after 14 weeks' gestation. When fetal anomalies were diagnosed the patients were informed about the possibilities of terminating the pregnancy or continuing the work-up and follow-up. Overall, ascertainment of fetal outcome was available in 95% of the study population.

Results

We performed 6223 NT examinations during the study period (2005-2011). The fetal anomalies detected included the following: seven skeletal anomalies, eleven brain anomalies, eight urinary system anomalies, nine abdominal anomalies, and three facial anomalies. 20 of 38 patients chose to discontinue the pregnancy shortly following detection of the congenital anomaly (within 14 weeks' gestation) and 11 patients waited for a repeated confirmatory scan to establish the diagnosis. Additional 34 non cardiac structural fetal anomalies were detected following the anatomy scan performed at 14-16 or 22-24 weeks' gestation.

Conclusion

The opportunity to diagnose structural fetal anomalies in early pregnancy, justifies the approach of extended NT examination.

S-020

Measuring Free RNA in Maternal Circulation to Non-Invasively Profile the Placental Transcriptome: A Novel Clinical Test of Placental Function. Clare Whitehead, Sue Walker, Martha Lappas, Stephen Tong. *Department of Obstetrics and Gynaecology, Mercy Hospital, University of Melbourne, Heidelberg, Australia.*

Background:

RNA from the placenta is steadily released into the maternal circulation. Therefore, we hypothesised measuring these RNAs in maternal blood could be used to non-invasively profile the placental transcriptome, identifying the presence of significant placental hypoxia, apoptosis, and dysregulated growth.

Objective:

To examine whether RNA in maternal blood is correlated with differential expression of hypoxic, apoptotic and growth genes in placenta.

Methods:

We measured RNA in maternal blood (Paxgene system) by Taqman PCR and correlated expression with RNA isolated from placenta. Samples (n=20-30 for all groups) of both maternal blood and placenta were obtained normal pregnancies and those complicated by severe preterm fetal growth restriction and severe early onset preeclampsia. We reasoned pregnancies complicated by severe, preterm preeclampsia and FGR would be associated with significant placental hypoxia, apoptosis and a dysregulation of growth genes.

Results:

Placentas complicated by both severe preterm FGR and preeclampsia were all associated with increased placental hypoxia, apoptosis and dysregulation of major growth genes compared to controls (two sets of control placentas: preterm spontaneous labour, and term placentas). Importantly, there was complete correlation of upregulation of transcript abundance in both placenta and maternal blood for all 15 genes analysed. These genes have major roles in three fundamental biological systems [4 genes associated with the hypoxic response (HIF1, HIF2, adrenomedullin, lactate dehydrogenase); 6 genes that regulates growth (IGF1, IGF2, IGF1R, IGFBP2, GH2, ADAM12); 5 genes that regulate apoptosis (BAD, Bcl-2, Survivin, Bcl-xl, BIM)].

Conclusion:

RNA transcript abundance of genes regulating fundamental biological processes in maternal blood are strongly correlated with placental transcriptome in the setting of severe obstetric disease. It may form the basis of a novel non-invasive

test that allows serial monitoring of feto-placental health in pregnancies complicated by severe FGR or preeclampsia, allowing clinicians to better time delivery of such fetuses.

S-021

Opinions and Practice Patterns of Obstetricians-Gynecologists Regarding Amniocentesis in Twins. Joy Vink,[1] Britta Anderson,[2] Karin Fuchs,[1] Jay Schulkin,[2] Mary E D'Alton.[1] [1]*Obstetrics & Gynecology, Columbia University Medical Center;* [2]*Research Department, American College of Obstetrics & Gynecology.*

OBJECTIVE: An accurate amniocentesis-related loss (ARL) rate in twin gestations, including monochorionic/diamniotic (MCDA), remains elusive due to limited data & varying definitions of ARL. We examined practice patterns & attitudes of OB/GYNs regarding amniocentesis in twins & how they define/counsel their patients about ARL.

METHODS: 347 American College of OB/GYN Collaborative Ambulatory Research Network members were mailed surveys about their opinions & practice patterns regarding amniocentesis in twins. 44% (152/347) of surveys were returned.

RESULTS: Of the respondents: mean age = 53 years, 52% were female, 84% were general OB/GYNs, & 10% were in Maternal Fetal Medicine. 18% performed amniocentesis in twins. 75% of all respondents counsel their patients with twins about an amniocentesis for prenatal diagnosis but 52% do not discuss an ARL rate. Of those who discuss ARL rates: 1) 69% quote an ARL rate > in singletons (most commonly used rates = 1/300 (44%) & 1/100 (27%)) 2) 45% quote a different ARL rate depending if 1 or both sacs are sampled (most commonly used rates were 1/300 for 1 sac (46%), 1/100 both sacs (45%)). Of the 10% who perform amniocentesis on MCDA twins, 39% state the ARL rate is =, 50% state > and 11% state < for dichorionic twins; of those that said the MCDA loss rate was >, 44% quote an ARL rate of 1/100. The most common clinical definitions & time intervals used to describe ARL are listed in Table 1.

Definitions & Time Intervals Used to Describe ARL

3 Most Common Clinical Definitions Used to Describe ARL	**%**
Preterm premature rupture of membranes (PPROM) of 1 or both sacs, preterm delivery (PTD) of 1 or both fetuses &/or intrauterine demise (IUFD) of 1 or both fetuses	51%
PPROM of 1 or both sacs	16%
PTD of 1 or both fetuses &/or IUFD of 1 or both fetuses	6%
3 Most Common Time Intervals Used to Identify Pregnancy Loss Due to an Amniocentesis	
Loss 2 weeks post-amniocentesis	51%
Loss 24 hours post-amniocentesis	13%
Loss 4 weeks post-amniocentesis	12%

CONCLUSION: Various definitions & rates of ARL are used when counseling patients about ARL in twins. Further studies & continued physician education using a widely accepted definition of ARL are necessary to improve the counseling of women carrying twin gestations who are considering an amniocentesis for prenatal diagnosis.

S-022

SOD1 Suppresses Maternal Hyperglycemia-Increased INOS Expression and Consequent Nitrosative Stress in Diabetic Embryopathy. Hongbo Weng,[1,2] Xuezheng Li,[2] E Albert Reece,[2] Peixin Yang.[2] [1]*Pharmacology, School of Pharmacy, Fudan University, Shanghai, China;* [2]*Obstetrics, Gynecology & Reproductive Sciences, University of Maryland School of Medicine.*

Oxidative stress mediates the teratogenicity of maternal diabetes. Hyperglycemia enhances the expression of inducible nitric oxide synthase (iNOS) and, thus, induces nitrosative stress. We hypothesized that oxidative stress is responsible for hyperglycemia-induced iNOS, and the resultant nitrosative stress leads to apoptosis in diabetic embryopathy. **Methods:** iNOS-luciferase activities, nitrosylated protein, lipidperoxidation markers 4-HNE and MDA were determined in PYS-2 cells (mouse embryonic yolk sac cell line) exposed to 5 mM glucose or 25 mM glucose with or without SOD1 (copper zinc superoxide dismutase 1) treatment. Levels of iNOS protein and mRNA, nitrosylated protein, cleaved caspase 3 and 8 were assessed on day 8.75 embryos from non-diabetic control, diabetic mellitus Wildtype and diabetic SOD-1 transgenic dams. **Results:** *in vitro* SOD1 treatment diminished high glucose-induced oxidative stress, as evidenced by reductions in 4-HNE and MDA. SOD1 treatment also blocked high glucose-increased iNOS expression, iNOS-luciferase activities, nitrosylated protein and lipidperoxidation. *in vivo* SOD1 overexpression suppressed hyperglycemia-increased iNOS expression and nitrosylated protein, and blocked cleavage of caspase 3 and 8. **Conclusions:** These data provide new evidence that diabetic embryopathy results from a cascade of events, including hyperglycemia-induced oxidative stress, which, in turn, induces iNOS gene expression, nitrosylated stress and apoptosis.

S-023

Hyperleptenima in Early Gestation Induces Fetal Programming Events in Female Offspring in Mice. Kathleen A Pennington, Ashley Sigafoos, Lindsey Beffa Martin, Laura Clamon Schulz. *Ob-GYN and Women's Health, University of Missouri, Columbia, MO, USA.*

Introduction: Obesity is a growing epidemic. Children born to either underweight or overweight mothers have a higher incidence of adult obesity and diabetes. This phenomenon is referred to as fetal programming. One potential mediator of nutritional fetal programming is leptin, a critical regulator in the satiety pathway that controls energy balance and food intake. People with increased BMI often develop hyperleptinemia and leptin resistance, whereas those who are undernourished have decreased circulating leptin. The goal of this work is to elucidate the role of leptin in fetal programming.

Methods: Pregnant mice were randomly placed in one of three treatment groups: *ad libitum* feed plus saline injection (control, n=5), 50% food restriction plus saline injection (restricted, n=4) or 50% food restriction plus 1mg/kg/day leptin injection (leptin, n=4). Mice were treated from dpc 1.5 to 11.5 and then returned to *ad libitum* feeding until weaning. At 19 weeks post weaning, offspring were placed on a 45% fat diet and then followed until 26 weeks post weaning, at which time they were sacrificed and samples were collected for further analysis

Results: Maternal serum leptin concentrations were significantly increased ($p<0.05$) in the leptin treated mothers compared to control and restricted mothers at dpc 11.5 but were not different at dpc 18.5. Female offspring born to leptin-treated mothers had significantly higher ($p<0.05$) body weights from week 20 post weaning until sacrifice versus control and restricted offspring. Body fat percentage was significantly increased ($p<0.05$) in female leptin offspring versus restricted but not different from control. Serum insulin concentrations were significantly increased ($p<0.05$) in female leptin offspring versus control and restricted. Glucose tolerance trended to be impaired in female leptin offspring compared to control and restricted female offspring. Immunhistochemistry of pancreatic islets revealed that female offspring from leptin and restricted mothers tended to have increased β cell number ($p=.11$) and β cell mass ($p=.12$) compared to control female offspring.

Conclusions: Our results indicate that high leptin exposure during early gestation can cause fetal programming events in female offspring, including obesity and insulin resistance. These effects may mimic fetal programming events seen in offspring of overweight women. Work supported by NIH HD055231.

S-024

Fetal Arterial Function Is Altered in the Placenta-Specific *Igf2* Knockout Mouse. Lewis J Renshall, Mark R Dilworth, Bernadette C Baker, Susan L Greenwood, Colin P Sibley, Mark Wareing. *Maternal and Fetal Health Research Centre, The University of Manchester, United Kingdom.*

Introduction: Neonates that do not reach predetermined genetic growth potential are at increased risk of cardiovascular disease in adulthood[1]. The placenta specific insulin-like growth factor II *(Igf2)* knockout mouse (P0) exhibits fetal growth restriction (FGR): >95% of P0 pups were <5th centile for body weight compared with wild-type (WT)[2]. We hypothesised that vascular function may be altered in such fetuses. To test this hypothesis we assessed fetal vascular function using the descending aorta and compared function between WT (normally grown) and P0 (FGR) siblings.

Methods: Fetal descending aortas were dissected from pups at E18.5. Vessels were mounted on a wire myograph, normalised at 0.9 L5.1kPa, equilibrated (20min; 37ºC; air/5% O2) and contractility assessed (120mmol KCl; 10μM phenylephrine (PE); constriction-response curves to the thromboxane A2 mimetic U46619 [0.1-2000nM]). Endothelial-dependent and -independent relaxation was assessed in pre-constricted vessels (U46619 EC_{80}) using acetylcholine (ACh, 0.1-10000nM) and sodium nitroprusside (SNP: 0.1-10000nM), respectively. Data are expressed as median (range). Number of litters (N) = 5; number of fetuses (n) = 16 (WT), 7 (P0).

Results: Aortic diameter was similar in P0 vs. WT [648 (552-737) vs. 653 (592-798) μm respectively; $P>0.05$ Mann-Whitney U (MWU) test]. Maximal constriction to KCl, PE and U46619 was also similar in P0 vs. WT [$P>0.05$; MWU test]. The U46619 dose-response curve (as a % of KPSS) was significantly shifted downward for P0 vs. WT [$P<0.05$; 2-way ANOVA] but sensitivity was not affected [EC_{50}: 7 (3-25) vs. 9 (4-41) nM respectively; $P>0.05$; MWU test]. ACh-induced relaxation was significantly shifted downward [$P<0.05$; 2-way ANOVA] but sensitivity was not affected [EC_{50}: 49 (1-217) vs. 133 (3-376) nM respectively; $P>0.05$; MWU test]. SNP-induced relaxation was similar in P0 vs. WT pups [$P>0.05$, 2-way ANOVA].

Conclusion: These data suggest that, in a mouse model of FGR with an abnormally functioning placental exchange barrier[3], fetal vascular function is compromised. We speculate that this abnormality may persist into adulthood. This novel technique may be used in future to assess cross generational effects of pregnancy complications using other appropriate mouse models.

[1]Osmond *et al* 2000 *Env Health Perspect*;108:545.
[2]Dilworth *et al* 2011 *Placenta* (In press).
[3]Kusinski *et al* 2011 *Placenta*. (In press).

S-025

Determining the Effect of Preeclampsia on Growth and Differentiation Potential of Umbilical Cord Blood Hematopoietic Stem Cells. Donna A Santillan, Wendy S Hamilton, Mark K Santillan, Stephen K Hunter. *Obstetrics & Gynecology, University of Iowa Hospitals & Clinics, Iowa City, IA, USA.*

Objective:
Late pre-term infants from preeclamptic pregnancies have higher rates of neonatal intensive care unit admission, small for gestational age, and longer hospital stay than infants from normotensive pregnancies. Differences have been reported in cytokine levels, nucleated red blood cell (nRBC), and neutrophil counts between children born to preeclamptics controls. The objective of this study is to compare the *in vitro* growth and differentiation capability of CD34+ umbilical cord blood hematopoietic progenitor cells from preeclamptic and control pregnancies.

Methods:
Umbilical cord blood was obtained from 9 preeclamptic and 5 normotensive women from the Institutional Review Board approved Maternal-Fetal Tissue Bank at the University of Iowa Hospitals & Clinics. CD34+ cells were isolated by immunomagnetic selection (Stem Cell Technologies) and grown in duplicate in a methylcellulose-based colony assay. After 16 days, colonies were identified by morphology and enumerated.

Results:
No differences were found in umbilical cord blood volume or CD34+ cell counts. We evaluated the differences in the colony assay between CD34+ umbilical cord blood cells obtained from normotensive women (n=5) and mild (n=5) and severe (n=4) preeclamptics. No significant differences were found in the number and types of colonies; however, there was a trend toward increased burst forming units-erythroid (BFU-E) in the preeclamptic samples versus normotensive samples. This trend of increased BFU-E colonies was observed in severe versus mild preeclamptics. Subjectively, smaller colonies were repeatedly observed in the preeclamptic samples.

Conclusion:
Little is known regarding the effects of preeclampsia on hematopoietic stem cells (HSC). Our preliminary results do support the findings of increased nRBCs and suggest that preeclamptic fetal HSCs more readily differentiate along the erythroid pathway. The hypoxic placental microenvironment in preeclampsia may be affecting the growth and differentiation potential of CD34+ hematopoietic progenitor cells. This compensation for hypoxic conditions by generating more erythoid cells and less immunoprotective cells supports the observation that neonates from preeclamptic pregnancies may be more prone to infection.

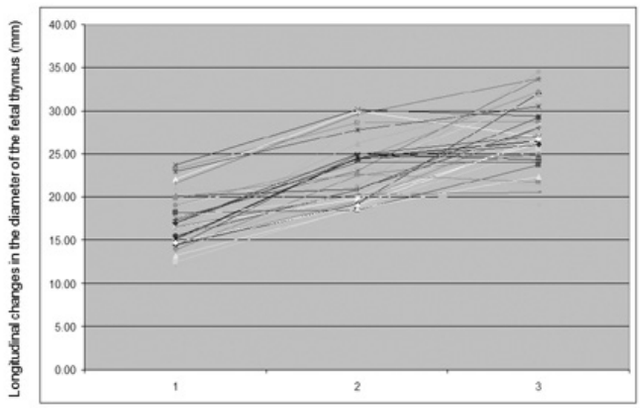
S-026

Feasibility of Serial Sonographic Fetal Thymic Measurements. Juan De Leon,[1] Joaquin Santolaya-Forgas,[2] Francisco Gamez,[1] Pilar Pintado,[1] Jacobo Santolaya,[2] Ricardo Perez,[1] Luis Ortz-Quintana.[1] *[1]Obstetrics and Gynecology, Gregorio Maranon General Hospital, Madrid, Spain; [2]Obstetrics and Gynecology, UMDNJ_Robert Wood Johnson Medical School, New Brunswick, NJ, USA.*

Objectives: The thymus allows for T-cell maturation. Cross-sectional ultrasound (US) and Magnetic Resonance Imaging studies have demonstrated that congenital agenesis of the thymus and thymic hypoplasia can be diagnosed during the antenatal and postnatal periods. The utility of US to determine progressive fetal thymic involution in response to intrauterine stress, however, remains to be determined. Our aim was to determine the feasibility of serial fetal thymic measurements.

Study Design: This was a prospective-nested US study in which the fetal thymus was measured in 40 uncomplicated singleton pregnancies at 3-time-points in gestation using a validated method (Zalel et al, 2002).

Results: The transverse diameter of the fetal thymus was measured in all cases. Figure 1 depicts the trend of growth of the thymus in each fetus (Y-axis = Transverse Diameter of the fetal thymus in mm. X-axis = time points for US measurements between 24 and 38 weeks gestation).



Conclusion: Our findings suggest that longitudinal US measurements of the fetal thymus are feasible which opens the opportunity for new research concerning the progressive effect of pathologic states of pregnancy on the development of the fetal thymus and neonatal and children T-cell function.

S-027

Developmental Programming of Obesity. Isaac Sasson, Monica Mainigi, Rebecca Simmons. *OB-GYN, UPENN, Philadelphia, PA, USA.*

Numerous studies in humans and animal models show that maternal obesity is associated with subfertility, intrauterine growth retardation, and an increased risk of metabolic abnormalities in the offspring. However, it is not known if the critical window of exposure occurs prior to (oocyte) or during in-utero development. We hypothesized that the phenotypes observed in the offspring of obese dams were due to the effects of maternal obesity on the oocyte prior to pregnancy. As previously reported by others, oocyte quality was significantly poorer in diet induced obese (DIO) dams compared to controls. The number of oocytes that failed to undergo GVBD was 25% in DIO dams vs. 4% in controls (p0.05). In addition, 10% of DIO oocytes were degenerated vs. 0% in controls (p0.05). Expression arrays in DIO oocytes showed differential expression of genes regulating pathways related to chromatin remodeling, RNA processing, mitochondrial metabolism, and embryonic growth. Two-cell embryos were generated by mating DIO dams (35.9g) to wild-type males (GFP-) and control dams (20.6g) to males expressing GFP under a ubiquitous promoter (GFP+). Similar numbers of each type of embryo were transferred together into a DIO or control pseudo-pregnant recipient. When delivered from a control recipient, DIO-derived pups had lower fetal and placental (e12.5) and neonatal weights than siblings derived from a control-dam (p0.001). Pre-implantation exposure to a high fat diet had no effect on adult weight in female progeny; however, animals born to a DIO carrier were significantly larger than animals delivered to a control carrier regardless of their pre-implantation exposure (p0.01). Progeny of the DIO carrier displayed impaired glucose tolerance (IGT) compared to progeny of the control carrier (p0.05). When carried by a DIO recipient, the control-derived progeny displayed a significantly greater IGT than the DIO-derived siblings (p0.05). Exposure of the oocyte to obesity affects the resulting embryo such that progeny have decreased fetal, placental, and neonatal weight. By contrast, adult weight in female progeny is impacted by maternal weight during pregnancy rather than pre-implantation embryonic exposure. Exposure of the post-implantation embryo to maternal obesity results in IGT. This IGT is exacerbated when there is a mismatch between the pre-implantation and post-implantation exposures. These data suggest that pre-implantation exposure to a maternal HFD programs how the resulting progeny utilize nutrient resources.

S-028

Enhanced Mesenteric Arterial Responsiveness to Angiotensin II in Prenatally Protein Restricted Adult Offspring Is Reversed by Flutamide. K Sathishkumar, Vijayakumar Chinnathambi, Meena Balakrishnan, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*

Maternal protein restriction results in intrauterine growth restriction (IUGR) and hypertension in adult female growth-restricted rats. Blockade of the renin-angiotensin system (RAS) abolishes hypertension in adult growth-restricted rats, suggesting that the RAS contributes to IUGR-induced hypertension. Moreover, the growth-restricted adult rats have higher plasma testosterone levels and anti-androgen (flutamide) treatment abolishes hypertension, indicating an important role for testosterone. Therefore, we hypothesized that

enhanced responsiveness to angiotensin II (Ang II) contributes to hypertension in this model of IUGR and that androgens may play a pivotal role in this enhanced response.
Methods: Pregnant rats were fed with 20% protein (control) or 6% protein (low-protein (LP) diet). Female offspring at 6 months age, were treated with androgen receptor antagonist, flutamide (30 mg/kg/day; SC), or its vehicle daily for 10 days, following which mean arterial pressure (MAP) and vascular reactivity were assessed.
Results: Baseline MAP in the vehicle-treated LP offspring was significantly higher compared to controls (1133.6 mm Hg vs. 982.4 mm Hg; $P<0.05$). Flutamide treatment reduced MAP in LP offspring (1051.9 mm Hg) but was without significant effects in controls (105 1.5 mm Hg). Enhanced contractile response to angiotensin II in mesenteric arteries was observed in vehicle-treated LP offspring (logEC50 = -10.160.10) compared with control (logEC50 = -9.490.08). Flutamide treatment reversed the enhanced contractile response to angiotensin II in LP offspring (logEC50 = -9.180.14) with no significant effect in controls (logEC50 = -9.290.14). Vascular reactivity to phenylephrine was similar between the control and LP offspring with and without flutamide treatment, suggesting that enhanced contractile response and flutamide's reversal effect is specific to angiotensin II. Vascular AT_1R/AT_2R ratio was significantly higher in LP offspring; an effect that was reserved by flutamide. Flutamide treatment did not have any effect on AT_1R/AT_2R ratio in controls.
Conclusion: These results suggest that prenatally protein-restricted rats exhibit an enhanced responsiveness to angiotensin II that is testosterone dependent and indicate that the RAS may serve as an underlying mechanism in mediating hypertension programmed in response to maternal protein restriction.

S-029

Advanced Maternal Age Increases the Risk of Congenital Heart Defects in Offspring through an Oocyte-Independent Mechanism. Claire E Schulkey, Suk D Regmi, Patrick Y Jay. *Pediatrics, Washington University School of Medicine, St. Louis, MO, USA.*
Introduction: Epidemiologic studies commonly report a correlation between maternal age and the risk of congenital heart defects (CHD) even after excluding chromosomal anomalies. The mechanistic basis is commonly presumed to be related to oocyte quality. Ongoing work in our laboratory has mapped genetic modifiers of phenotype in a mouse model of CHD. Examination of a large dataset from one inbred mouse strain cross revealed the independent effect of maternal age.
Hypothesis: The increase of CHD incidence in offspring of old mothers is due to a maternally intrinsic or oocyte intrinsic effect.
Methods: We have phenotyped >3000 *Nkx2-5*$^{+/-}$ mouse pups in an F2 intercross of C57BL/6 and FVB/N. In addition to Nkx2-5 genotype, maternal and paternal age and litter size are known for every pup. Multiple logistic regression was performed to determine the effect of these non-genetic factors on the risk of ventricular septal defects (VSD). Copy number variation at SNPs spaced 10-20 cM across the genome was assessed using the R.sqnm algorithm on Sequenom mass spectroscopy data. To localize the maternal age effect, we performed reciprocal ovarian transfers between old and young mothers. The resulting offspring were collected on the day of birth, genotyped, and diagnosed for heart defects.
Results: The risk of VSD in *Nkx2-5*$^{+/-}$ but not wildtype pups is strongly correlated with maternal age resulting in a doubling of risk from early to late reproduction. Thus, a factor related to oocyte or maternal age modifies risk but does not cause disease. Chromosomal aneuploidy was not detected in 250 animals, ruling out meiotic nondysjunction as the factor. In ovarian transplant experiments between old and young mothers, the incidence of CHD in the offspring tracked with the age of the mother and not the transplanted ovary ($p < 0.01$). The age of the oocyte has no discernable effect on the incidence of CHD.
Conclusions: Maternal age is correlated with the risk of congenital heart disease in human and a mouse model. Our results localize the basis of the effect to a maternal factor that affects embryonic cardiac development. Ongoing experiments seek to characterize the pathophysiologic and epigenetic basis of the maternal age effect.

S-030

Maternal and Newborn Fatty Acid Elongation Activity in Obese Pregnancy. Emily Seet,[1] Jennifer Yee,[2] Michael G Ross,[1] Mina Desai.[1] *[1]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Pediatrics, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
OBJECTIVE: Obesity in pregnancy has not only adverse effects on maternal health and pregnancy outcome but also on the developing fetus. Offspring born to mothers with a high body mass index (BMI) are often larger at birth, and show increased adiposity and lipid abnormalities in later life. As the prevalence of obesity among pregnant women continues to rise, increasing number of children are exposed to an 'obese intrauterine environment' during development. We have established a rat model of maternal obesity that results in normal birth weight newborns that develop obesity at 3 weeks of age and continue in adulthood. Elongated (C18) and desaturated fatty acids are the preferred substrate for triglyceride synthesis and fat deposition. To assess the potential for programmed adiposity, we quantified the fatty acid elongation index (EI; C18/C16) and desaturation index (DI; ratio of desaturated to saturated fatty acids) in obese pregnant dams and their newborns.
METHODS: At 3 weeks of age, female rats were weaned to high fat (Obese: 60% k/cal) or (Control, 10% k/cal) diet. At 11 weeks of age, rats were mated and continued on their respective diets during pregnancy and lactation. Blood samples were obtained from Obese and Control dams at term (day 21) and from newborn males at 1 day of age. Plasma samples were saponified, fatty acids extracted, and GC/MS performed. GC/MS peaks were utilized to compute EI (stearate/palmitate), and DI for C18 (oleate/stearate) and C16 (palmitoleate/palmitate) in dams and offspring. Values are means±SE.
RESULTS: At term, Obese dams had significantly higher body weights (430±17 vs. 330±5 g; $p<0.01$) with comparable triglyceride levels to Controls. The EI was significantly increased in both Obese dams and their offspring (0.92±0.03 vs 0.78±0.02; $p<0.01$ and 1.06±0.04 vs. 0.84±0.05; $p=0.05$, respectively). In contrast, Obese dams exhibited significantly lower DI (C18 and C16 fatty acids) while newborns of Obese dams had significantly lower plasma triglycerides levels (92 ± 12 vs 126 ± 9mg/dl) and lower DI (C16 fatty acids).
CONCLUSION: The increased EI of both Obese dams and offspring suggests enhanced potential for adiposity. We propose that hepatic fatty acid elongation combined with local adipose desaturation activity is responsible for enhanced adiposity, rather than circulating triglyceride levels.

S-031

Maternal Undernutrition during Different Developmental Windows Is Associated with Altered Ovarian Core Clock Gene Transcription Factors and Impaired Follicle Growth. Angelica Bernal,[1] Mark H Vickers,[1,2] Deborah M Sloboda.[1,2] *[1]Liggins Institute, University of Auckland, Auckland, New Zealand; [2]The National Research Centre for Growth and Development, University of Auckland, New Zealand.*
OBJECTIVE: Functional circadian rhythmicity, regulated by transcription/translation feedback loops and clock genes such as *Bmal1*, *Clock*, period (*per1*, *per2*) and cryptochrome (*cry1*, *cry2*), is present in peripheral organs such as the ovary. These signaling pathways are required for optimal reproductive outcomes; disruptions in ovarian clocks leads to impaired reproductive function. We have shown that offspring born to undernourished mothers enter puberty early and have impaired ovarian function, reduced ovarian follicle numbers, and increased ovarian oxidative stress. We hypothesised that changes in ovarian clock gene transcription and apoptotic signaling contribute to impaired folliculogenesis and may result in premature ovarian ageing.
METHODS: Using a rat model, pregnant dams were randomized to a standard diet throughout pregnancy and lactation (Cont), or a calorie-restricted (50% of control) diet during pregnancy (UNP), during pregnancy and lactation (UNPL) or during lactation alone (UNL). At 160 days of age, offspring ovaries were collected and mRNA levels of core clock genes (*Bmal1*, *Clock*, *per1*, *per2*, *cry1*, *cry2)* and apoptotic markers *(Bcl2, Bax, casp3)* were determined using qPCR.
RESULTS: Maternal UN significantly altered ovarian mRNA levels of clock genes and apoptosis markers in offspring. Offspring of UNP mothers had increased ovarian *Bmal1, per1* but not *per2* mRNA levels compared to Cont. *Cry1* but not *cry2* was increased in UNP offspring; whereas UNPL and UNL offspring had increased ovarian *Clock* but not *Bmal1* mRNA levels, associated with higher *cry1* levels. All UN offspring had decreased ovarian Bax:Bcl2 expression ratio and casp3 mRNA levels.
CONCLUSIONS: These data suggest that maternal UN influences offspring ovarian core clock gene mRNA levels and these changes were dependent upon the timing of UN. Disruptions in ovarian circadian regulation in UN offspring may explain our previously observed changes in ovarian follicle number and increased oxidative stress. It appears that a loss of follicle number may be attributable to reduced follicular driver of growth and does not appear to be mediated by increased apoptosis. Changes in ovarian clocks may be one mechanism underlying the link between early life nutritional compromise and impaired reproductive function.

S-032

Prenatal Cocaine Exposure and Risky Sexual Behavior in Late Adolescence. R Sokol,[1] V Delaney-Black,[2] L Chiodo,[2] J Hannigan,[3] G Patterson,[2] J Janisse,[4] R Patridge,[5] M Greenwald,[6] S Ondersma,[1,6] L Lewandowski,[7] J Ager.[4] *[1]ObGyn, WSU; [2]Peds, WSU; [3]ObGyn, MPSI/WSU; [4]Family Med, WSU; [5]Psychol, WSU; [6]Psychiat, WSU; [7]Nursing, WSU.*

Background: We previously reported a relation between prenatal cocaine exposure (PCExp) and externalizing behavior (ExtBeh) in early adolescence. Early teen ExtBeh is an antecedent of "high-risk" behaviors in late adolescence such as the initiation and progression of illicit drug/alcohol use and involvement in sex-risk behaviors. To date there are no published data linking PCExp to these critical adolescent behaviors.

Objective: To examine the impact of PCExp and teen cocaine use (Coc) on "high-risk" late adolescent risky sexual behavior (RSexBeh).

Method: PCExp was assessed prospectively at an urban antenatal clinic. Based on self-report and biological measures, PCExp was categorized as 'None' (N=130), 'Some' (N=37) or 'Heavy and/or Persistent' (N=57; ≥2 times/week or continued to term). At follow-up visit (mean age=19.6; SD=0.4; N=224; 43.2% male) teens completed the Sexual Risk Behavior Scale (Jemmott et al, 1999) and the Sexual Knowledge and Attitudes Test for Adolescents (SKAT-A, Lief et al., 1990). Risk variables were examined including contraception use, forced sexual activity and promiscuity. A biomarker of cocaine use (hair analysis) was used at the 14-yr visit and in the analyses.

Results: In univariate analyses, RSexBeh was correlated with both PCExp and the 14-year biomarker of teen Coc. Teens with heavy/persistent PCExp reported less contraception use (r=-0.15*), specifically less condom use (r=-0.16*), higher frequency of sexual activity after drinking (r=0.17**) or using drugs (r=0.16*), or after their partner had been drinking (r=0.20**). Any 14-yr Coc use significantly predicted having sex with a partner who was also having sex with someone else (r=0.14*). Teens with any Coc use talked more about HIV/AIDS and sexual activity with partners than teens who did not use Coc (r=0.16*). Early teen Coc use predicted engaging in sexual activity to get food/clothing (r=0.16*) and forcing a sexual partner to have sex (r=0.17*). [*=p<.05; **=p<.01].

Conclusion: Both PCExp and teen Coc use are associated with significant high-risk behaviors. While the mechanism(s) have yet to be explicated, these findings are consistent with the concept of fetal origins of adult disease and suggest that adverse consequences even later in life would not be surprising.

S-033

Gender Differences in the Effect of Antenatal Betamethasone (Beta) Exposure on Sodium Uptake in Ovine Renal Proximal Tubule Cells (RPTC). Yixin Su, Lijun Tang, Jianli Bi, Jorge P Figueroa, James C Rose. *Obstetrics and Gynecology, Wake Forest University Health Science, Winston Salem, NC, USA.*

Prenatal exposure to elevated levels of glucocorticoids reduces nephron number and alters the intrarenal renin-angiotensin system in animals. These changes in early life may have untoward consequences in adult offspring. The present study was designed to examine whether antenatal betamethasone exposure at 80-81 days of gestation would impact sodium uptake by RPTC from adult ewes and rams at 1year of age. RPTC were isolated from 1-yr-old male (3) and female (N=7) vehicle-treated and male (7) and female (N=9) Beta-treated (N=7) offspring and cultured for 7-10 days. The fluorescence dye sodium green was employed to determine cytoplasmic Na+ uptake. In the presence of the Na+/K+ ATPase inhibitor ouabain, Na^+ uptake was evaluated in RPTC exposed to different Na^+ concentrations from 30 to140 mmol/L alone or in the presence of angiotensin II (AngII). The cellular Na^+ uptake was expressed as the percent change of fluorescence emission of the dye monitored at the wavelength (excitation 507 nm, emission 532 nm). There were gender (male>female, F=25.1, p<0.001) effects seen in unstimulated Na^+ uptake. There were also gender effects (male > female, F=56.2, P<0.001 in AngII stimulated Na^+uptake. Antenatal Beta increased basal (F=4.1, P<0.05) and AngII stimulated (F=5.65, P<0.05) uptake by cells from males, but not from females. These data suggest that there are gender specific effects on Na^+ uptake by RPTC and that females are protected from the effects of prenatal Beta exposure on RPTC Na^+ uptake. Supported by NIH grants HD 17644 and HD 47584.

S-034

Intrauterine Origins of Future Adult Development: Improving Reading Ability among Young Children with Poor Reading Skills Depends on Perinatal Track Record. Jacoba van der Kooy,[1] Verna AC van der Kooy,[2] Adriana G Bus,[2] Gouke J Bonsel.[1] *[1]Erasmus MC, Obstetrics and Gynaecology, Netherlands; [2]Leiden University, Education and Child Studies.*

INTRODUCTION

The incidence of perinatal adverse outcome is increasing in urban environments. Children born with adverse perinatal outcomes show delay in cognitive development, e.g., poor language and reading skills.

The fetal origins hypothesis events not only may have a profound impact on one's risk for future adult disease or impaired psychomotor development, but also might influence the plasticity to improve upon specific interventions (medical, social, educational).

OBJECTIVE

To study differential improvement of early literacy skills following a computer-aided reading intervention in children with initial poor reading skills, depending on the presence of perinatal adverse outcomes.

METHOD

404 senior kindergarten Dutch speaking children were screened on early literacy skills. The 30% lowest performing children were selected for a computer-aided reading intervention. After consent detailed perinatal outcomes were obtained from the Netherland Perinatal Registry. Pre- and posttest literacy skills were obtained. The effect of the intervention was estimated by regression analysis, adjusted for parental educational level and child IQ.

RESULTS

The reading improvement intervention showed a significant and relevant improvement in the perinatal adverse outcome group only (p<0.001).

CONCLUSION

Fetal development and delivery not only determine successful psychomotor and future cognitive development such as expressed in reading skills, but also modify the capacity to improve if skills are low. This stresses the importance to recognize the perinatal background of children with delayed development. The initial disadvantage apparently can be turned from a possible handicap into a normal position in the presence of adequate support.

S-035

Maternal Inheritance of Cardiovascular Disease in Families and Congenital Heart Disease in Offspring: First Transgenerational Evidence. Kim PJ Wijnands,[1] Sylvia A Obermann-Borst,[1] Eric JG Sijbrands,[2] Mark F Wildhagen,[1,3] Willem A Helbing,[4] Regine PM Steegers-Theunissen.[1,5,6] *[1]Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Internal Medicine, Erasmus University Medical Center, Rotterdam, Netherlands; [3]Urology, Erasmus University Medical Center, Rotterdam, Netherlands; [4]Pediatrics, Division of Pediatric Cardiology, Erasmus University Medical Center, Rotterdam, Netherlands; [5]Epidemiology, Erasmus University Medical Center, Rotterdam, Netherlands; [6]Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands.*

Background

Hyperglycemia, dyslipidemia and hyperhomocysteinemia are features of a poor lifestyle and are associated with cardiovascular disease (CVD) risk. In mothers-to-be the same factors also enhance the risk of having offspring with a congenital heart disease (CHD).

Objective

To investigate associations between CVD in grandparents, as proxy for poor lifestyle, and the risk of having a grandchild with CHD.

Methods

In a case-control family study information on CVD and lifestyles was obtained through questionnaires from 250 families with and 211 families without a child with CHD.

Results

We found a higher prevalence of CVD in grandparents of children with CHD compared to the control grandparents (17% versus 13%, p-value 0.034). Maternal grandfathers of a child with CHD were treated more often for CVD and other aging diseases (72% versus 63%, p-value 0.038) and their fathers, i.e. great-grandfathers, showed more frequently CVD (44% versus 32%, p-value 0.015). It revealed that a child has a 1.7 times higher risk of CHD (95%CI 1.2-2.5) when one of the grandparents has CVD, even after adjustment for maternal risk factors (OR 1.6 (95%CI 1.04-2.4)). Moreover, a significant trend was observed for the number of affected grandparents (*p*-trend 0.002).

Conclusion

CVD in (great)-grandparents is associated with a 60% increased risk of grandchildren with CHD. Derangements in epigenetic programming of germ

cells by poor lifestyles may explain these transgenerational effects. This first finding emphasizes the importance of a healthy periconceptional lifestyle in mothers-to-be, but should be substantiated in large prospective studies.

S-036

Maternal Constitutional and Modifiable Factors for Perimembranous Ventricular Septal Defects in the Offspring: Implications for Preconceptional Care. Kim PJ Wijnands,[1] Gerda A Zeilmaker,[1] Willemijn M Meijer,[1] Willem A Helbing,[2] Regine PM Steegers-Theunissen.[1,3,4] *[1]Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Pediatrics, Division of Pediatric Cardiology, Erasmus University Medical Center, Rotterdam, Netherlands; [3]Epidemiology, Erasmus University Medical Center, Rotterdam, Netherlands; [4]Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands.*

Background

Perimembranous ventricular septal defect (pVSD) is the most common congenital heart disease (CHD). During the periconceptional period, several maternal factors are associated with pVSD in the offspring. We created a multifactorial risk factor model of maternal constitutional and modifiable factors to improve preconceptional counseling.

Methods

At 17 months after delivery of the child information on maternal factors was obtained through questionnaires from 113 case mothers and 480 control mothers. Data on age, parity, duration of pregnancy, mode of pregnancy, educational level, BMI, diabetes mellitus, ethnicity, family history of CHD, blood pressure, medication use and periconceptional use of folic acid supplements, alcohol and tobacco products were studied for each individual. To assess the 'predictive' value of maternal factors for the risk of pVSD, we applied logistic regression analysis with model building. Estimates of attributable risks of the maternal factors were calculated.

Results

The model including maternal age (OR=1.07, p=0.005), educational level (intermediate OR=0.92, p=0,833 / high OR=0.14, p=0.019), BMI (OR=0.609, p=0.445), family history of CHD (OR=3.06, p=0.000), folic acid supplement use (OR=1.63, p=0.214), medication use (OR=2.26, p=0.001) and interaction for educational level x folic acid supplement use was best in predicting the risk of pVSD in children (Hosmer-Lemeshow goodness-of-fit 0.724, Nagelkerke R^2 0.124). The attributable risk was highest for a low maternal education (89%).

Conclusion

Individual maternal risk factors for pVSD in the offspring are high age, low educational level, positive family history of CHD, folic acid supplement and medication use. These constitutional and modifiable factors 'predict' in 12.4% the chance of pVSD. A low maternal educational level attributed the most. Preconceptional counseling with an emphasis on reducing modifiable risk factors will contribute to the prevention of pVSD.

S-037

Histone Deacetylase Mediated Mechanism for Fatty Liver in Offspring of Obese Pregnant Dams. Diana Wolfe, Ming Gong, Guang Han, Michael G Ross, Mina Desai. *Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

OBJECTIVE: Nonalcoholic fatty liver disease (NAFLD) is associated with obesity and is currently a major cause of liver-related morbidity and mortality. The underlying mechanisms include altered hepatic lipid metabolism and gene expression leading to either increased fatty acid uptake, increased lipogenesis and/or decreased β-oxidation. Chromatin structure has a central role in the regulation of gene expression which occurs via modifications of histone deacetyltransferases (HDAC1). Specifically, studies in mice have demonstrated that overexpression of HDAC1 causes hepatomegaly and hepatic steatosis. We have shown that maternal obesity and high fat (HF) diet prior to and during pregnancy results in normal birth weight newborns. When nursed by HF dams, these offspring (HF) demonstrate early onset of obesity and fatty liver. We hypothesized that enhanced lipid accumulation in HF offspring is a result of gestationally programmed upregulated HDAC1. We measured hepatic protein expression of epigenetic modulator (HDAC1), nuclear receptor (RXRα), lipogenic transcription factor (SREBP1), lipolytic transcription factor (HNF4α) and lipogenic enzyme (fatty acid synthase) in 1 day old HF and Control male newborns.

METHODS: At 3 week of age, female rats were weaned to high fat (HF: 60% k/cal) or (control, 10% k/cal) diet. At 11 weeks of age, these rats were mated and continued on their respective diets during pregnancy. At 1 day of age, HF and Control male newborns were sacrificed and liver was collected. Hepatic protein expression (Western Blot) of HDAC1, RXRα, SREBP1, HNF4α and fatty acid synthase was analyzed. Values are expressed as fold change.

RESULTS: At 1 day of age, HF males exhibited significantly increased expression of hepatic HDAC1 (1.4-fold) and RXRα (1.3-fold). In addition, HNF4α (0.5-fold) and notably SREBP1 (0.6-fold) was downregulated whereas fatty acid synthase was upregulated (1.4-fold).

CONCLUSIONS: Increased hepatic HDAC1 expression prior to development of obesity suggests that HDAC1 mediated epigenetic mechanism contributes to programmed NAFLD in HF offspring of obese pregnant dams.

S-038

Antenatal Nicotine Gender-Dependently Attenuates Vascular AT_2R Gene Expression through DNA Methylation Mechanism in Adult Rat Offspring. DaLiao Xiao, Xiaohui Huang, Lubo Zhang. *Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Objective: Maternal smoking has adverse effects on fetal development and is associated with an increased risk of cardiovascular disease in offspring later in life. Our recent studies in rats have demonstrated that perinatal nicotine exposure increases blood pressure response to angiotensin II (Ang II) in male offspring in a sex-dependent manner. The present study tests the hypothesis that antenatal nicotine-induced vascular dysfunction is associated with an epigenetic modification of Ang II receptor type II (AT_2R) gene expression. **Methods:** Nicotine was administered to pregnant rats *via* subcutaneous osmotic minipumps throughout gestation and up to 10 days after birth. The aortic rings were isolated from 5-month-old adult offspring. The protein levels of AT_2R in the arteries were determined by Western blotting. The mRNA levels were determined by real-time RT-PCR, and CpG methylation of AT_2R gene promoter was measured by quantitative methylation-specific PCR. **Results:** Vascular AT_2R protein levels were significantly lower in female than those in male offspring. Antenatal nicotine significantly decreased the vascular AT_2R protein levels in male, but not in female offspring. Consistent with the protein expression patterns, the mRNA levels of AT_2R were also significantly lower in female than those in male offspring, and antenatal nicotine treatment attenuated the vascular AT_2R mRNA levels in male, but in female offspring. In addition, DNA methylation in the potential CpG methylation sites at AT_2R promoter region was detected by methylation-specific PCR analysis. Antenatal nicotine treatment significantly increased the aortic DNA methylation levels of AT_2R promoter at the CpG sites (-396 and +54), but not in the site of -4, as compared with the saline control in male offspring. **Conclusions:** The results indicate that antenatal nicotine causes a gender-dependent attenuation of vascular AT_2R gene expression, which is likely due to an increase in DNA methylation in AT_2R promoter at specific CpG sites. The nicotine-mediated decrease of AT_2R gene expression may play a key role in fetal programming of development of hypertensive phenotype in adult offspring (Supported in part by the California Tobacco-Related Disease Research Program Award 18KT-0024 and NIH R03DA032510).

S-039

Increased Propensity for Adipose Tissue Fatty Acid De Novo Synthesis Is Independent of Diet in SGA Adult Rats. Jennifer K Yee,[1] Juan Vega,[1] WN Paul Lee,[1] Michael G Ross,[2] Mina Desai.[2] *[1]Pediatrics, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

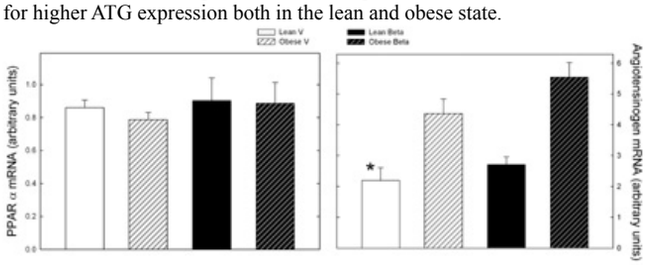
Objective: Small-for-gestational age (SGA) newborns are at increased risk for the development of adult obesity and insulin resistance. A gestationally programmed enhanced adipogenesis and lipogenesis are contributory mechanisms for obesity. Our rat model of maternal nutrient restriction results in SGA newborns, which when nursed normally exhibit obese phenotype. At 3 weeks of age, prior to onset of adiposity, SGA offspring demonstrate increased fatty acid de novo synthesis in adipose tissue, suggesting a gestationally programmed effect. As de novo lipogenesis may contribute to obesity, and a high fat diet is known to suppress de novo lipogenesis, we sought to determine if a high fat diet suppresses de novo synthesis in SGA adult offspring.

Methods: Control dams were fed normally from day 10 to 21 of gestation, and study dams were 50% food-restricted to produce SGA pups. All pups were nursed by Control dams and weaned to a standard diet (15% fat). At 6 months of age, Control and SGA male offspring were pair-fed either the standard diet (15% fat) or a high fat diet (30% fat) for two weeks. Offspring received drinking water with 6% deuterium as a stable isotope tracer for 14 days. Offspring were sacrificed, and the rate of production of the main product of de novo synthesis, palmitate, was determined in subcutaneous (SC) and retroperitoneal (RP) adipose tissue by GC/MS.

Results: On both high fat and standard diets, SGA intake was isocaloric (gm/kg/day) to Control intake. On the standard diet, SGA exhibited increased de novo synthesis of palmitate in SC (9.5±0.6 vs 7.5±0.8 %, $p<0.05$) and RP adipose tissue (12.6±1.5 vs 9.3±1.1 %, $p<0.01$) as compared to Control males. On the high fat diet, de novo synthesis was suppressed to similar levels in both groups in both SC (GA 3.3±0.3 vs Control 4.5±0.5 %) and RP adipose tissues (SGA 4.4±0.6 vs Control 4.2±0.4 %).
Conclusions: Adult SGA offspring exhibit increased adipose tissue de novo synthesis on a standard diet, but suppress normally in response to high fat. The mechanism of increased lipogenesis in this model, therefore, is likely related to increased activation of de novo synthesis, and not failure to respond to dietary fat availability. These findings suggest that restriction of dietary fat is not sufficient to prevent obesity in programmed offspring.

S-040

Effects of Antenatal Glucocorticoids and Diet-Induced Obesity on Adipose Tissue Renin Angiotensin System (RAS) in Adult Sheep. Jie Zhang, Angela G Massmann, Jorge P Figueroa. *Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, USA.*
Glucocorticoids (GC) play an important role in terminal differentiation of adipose tissue (AT) cells, in particular adipocytes. Therefore, we hypothesize that antenatal exposure to abnormally high concentrations of GC alters the development of white adipose tissue an ultimately AT function. Adipose tissue-derived inflammatory mediators and components of the RAS are thought to participate in the regulation of blood pressure in obese and diabetic humans.
OBJECTIVE: The aim of this study was to further characterize the alteration in adipose tissue expression of RAS components and to determine if superimposed obesity exaggerates the abnormalities found.
METHODS: Pregnant sheep were treated with two IM doses of betamethasone (Beta, 0.17 mg/kg) or vehicle (V) 24-h apart at 80 days gestation and allowed to deliver at term. At 9 mo of age, female sheep were randomly allocated to be fed at either 100% of nutritional allowance (Lean) or ad libitum for three months (Obese). At one year of age omental adipose tissue was harvested. AT protein and mRNA were extracted for determining expression levels of angiotensinogen angiotensin converting enzyme (ACE) and PPAR α. Data are expressed as Mean±SEM and were analyzed by ANOVA.
RESULTS: Ad libitum fed sheep gain > 50% of the original weight. Ometal fat expression of PPARα was not significantly affected by either antenatal GC or diet-induced obesity. In contrast, obesity significant increased ATG expression in both V and Beta sheep. Antenatal GC exposure was associated with a trend for higher ATG expression both in the lean and obese state.



CONCLUSION: Our data show that diet-induced obesity significantly activates AT RAS as demonstrated by a significant increase in ATG. Although not significant with the current sample size, the data is consistent with a long term of antenatal GC on AT function. Further studies are required to establish if this trend is indeed true. HL 68728 and HD 04784.

S-041

IUGR Increases Hepatic Cholesterol Levels without Changing SREBP2 and HMGCoA Reductase mRNA Levels in Female Rats Fed a High Cholesterol Diet. Erin Zinkhan,[1] Jeanette Chin,[2] Xing Yu,[1] Chengshe Jiang,[1] Christopher Callaway,[1] Lisa Joss-Moore,[1] Robert Lane.[1] *[1]Neonatology, University of Utah, Salt Lake City, UT; [2]Maternal Fetal Medicine, University of Utah, Salt Lake City, UT.*
Background: Intrauterine growth restriction (IUGR) increases the risk for hypercholesterolemia in adult humans and rats. A high cholesterol diet (HCD) worsens hypercholesterolemia in IUGR and non-IUGR humans and rats. Serum and hepatic cholesterol levels are regulated by sterol-responsive element binding protein 2 (SREBP2) via production of HMGCoA reductase (HMGCOaR). Hepatic cholesterol accumulation in turn decreases production of SREBP2. Despite the importance of SREBP2 and HMGCoAR in the regulation of cholesterol throughout life, little is known about the combined effect of a HCD and IUGR on hepatic cholesterol levels and SREBP2 and HMGCoAR mRNA levels in adulthood. We hypothesized that IUGR rats fed a HCD would develop increased hepatic cholesterol levels and decreased SREBP2 and HMGCoAR mRNA levels compared to HCD fed controls.
Methods: To test this hypothesis IUGR was induced by bilateral uterine artery ligation at day 19 of gestation. At weaning, control and IUGR rats were fed either a control diet (CD) or a high (2%) cholesterol diet (HCD). Lipid was isolated from liver at day of life 60 and cholesterol was quantitated with a colorimetric kit. Real-time RT PCR was used to determine mRNA levels of SREBP2 and HMGCoAR.
Results: IUGR and CD did not increase baseline hepatic cholesterol levels. HCD increased baseline hepatic cholesterol in males 165% ($p<0.01$) and females 238% ($p<0.01$). IUGR and HCD further increased hepatic cholesterol in females 157% ($p<0.001$) compared to HCD fed controls. IUGR and CD decreased female mRNA levels of SREBP2 54% ($p<0.01$) and HMGCoAR 50% ($p<0.001$). HCD decreased baseline mRNA levels of SREBP2 in males 59% ($p<0.01$) and females 48% ($p<0.0001$) and HMGCoAR in males 34% ($p=0.07$) and females 14% ($p<0.001$). IUGR and HCD did not further decrease SREBP2 and HMGCoAR mRNA levels.
Conclusion/Speculation: We conclude that IUGR increases hepatic cholesterol accumulation without changing SREBP2 and HMGCoAR mRNA levels in female rats fed HCD. IUGR alone does not change hepatic cholesterol accumulation but decreased SREBP2 and HMGCoAR mRNA levels in female rats fed CD. Therefore we speculate that IUGR bypasses normal transcriptional regulatory mechanisms in the setting of HCD.

S-042

Developmental Expression of Cell Cycle Regulators in the Primate Fetal Adrenal Cortex. Adina Maniu,[2] Gerald J Pepe,[2] Eugene D Albrecht.[1] *[1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD, USA; [2]Departments of Obstetrics and Gynecology and Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA, USA.*
We have previously shown that the volume of and formation of the C_{19}-steroid estrogen precursor dehydroepiandrosterone (DHA) by the fetal zone of the fetal adrenal cortex were increased by suppressing estrogen synthesis/levels during the second half of baboon pregnancy (Albrecht et al., Endocrinology 146:1737, 2005). We have suggested that estrogen represses the growth of the fetal zone in a feedback manner to maintain physiological levels of estrogen. Moreover, fetal adrenal expression of the cell cycle regulator cyclin D1 was decreased between mid and late gestation in association with the rise in estrogen (Dumitrescu et al., J Endocrinol 192:237, 2007) and in estrogen-suppressed baboons expression of cyclin D1 was increased and the cyclin-dependent kinases (cdk) 4 and 6 unaltered (Maniu et al., Soc Gyn Invest Abstract #1007, 2010). Activity of the cyclin/cdk complex is modulated by the regulatory proteins $p27^{Kip1}$ and $p57^{Kip2}$. In the present study, therefore, the mRNA levels for $p27^{Kip1}$ and $p57^{Kip2}$ were determined by real-time RT-PCR in whole fetal adrenal glands obtained on days 60 (early, n=4), 100 (mid, n=5) and 165 (late, n=5) of gestation in untreated baboons and day 165 in baboons treated with letrozole (0.115 mg/kg BW, sc, n=5) or letrozole plus estradiol (each at 0.115 mg/kg BW, sc, n=5) daily on days 100-164 of gestation. $P27^{Kip1}$ mRNA levels increased over 2-fold ($P<0.01$) between mid (0.45 ± 0.08) and late (1.19 ± 0.21) gestation, but were unaltered by letrozole (1.19 ± 0.16) or letrozole plus estradiol (1.41 ± 0.14) administration. In contrast, $p57^{Kip2}$ mRNA levels remained constant at mid (2.89 ± 0.21) and late (3.44 ± 0.48) gestation and were not changed by letrozole ± estradiol treatment. $P27^{Kip1}$ protein was localized by immunocytochemistry primarily in the outer definitive zone and $p57^{Kip2}$ in the fetal zone of the baboon fetal adrenal. We suggest that estrogen controls the growth and production of estrogen precursors by the fetal zone during primate pregnancy and cyclin D1, but not the cdk's or p27/p57 inhibitors, mediate this regulatory effect of estrogen. Supported by NIH R01 HD13294.

S-043

Fetal Brain MRI – Experiences in the Ovine Model of Cerebral Inflammatory Response (CIR). EN Carmel,[1] P Burns,[1] D Durosier,[2] C Duchatellier,[2] M Cao,[2] A Desrochers,[1] G Fecteau,[1] M Frasch.[2,3] *[1]FMV, U Montréal; [2]ObGyn & Res Centre, CHUSJ; [3]CRRA, U Montréal.*
BACKGROUND
In fetal sheep, variable systemic and CIR with regionally patterned neuronal injury can result from repetitive umbilical cord occlusions leading to severe acidemia, as might occur during human labour, as well as from lipopolysaccharide administered to fetus to model chorioamnionitis. We

Saturday

hypothesized that fetal sheep brain MRI would allow to visualize and quantify in vivo the development of CIR and neuronal injury. As first step, here we aimed at developing a robust protocol of fetal sheep brain MRI capable of visualizing CIR.

METHODS

Three near-term fetal sheep at 0.90 gestation were chronically prepared with vascular catheters and ECG. Brain MRI was performed in a 1.5T MRI at 0.94 gestation with the two sheep anesthetized and mechanically ventilated. Sequences used included transverse T2w single-shot FSE (SSFSE), T2w FSE, STIR, 3D SPGR and DWI with the addition of respiratory gating (RG) for the second animal. An ex-vivo MRI was performed on the brain of the third sheep fetus to document normal brain anatomy.

RESULTS

T2-w SSFSE sequences were a rapid means of evaluating fetal position in utero. T2w FSE images provided the best signal-to-noise ratio and good visualization of cerebral structures. However, a clear demarcation between gray matter (GM) and white matter (WM) was not obtained. STIR images provided better GM/WM distinction. Use of RG significantly improved image sharpness and contrast between the structures but increased acquisition time by 25%. T2w FSE images acquired with RG demonstrated the highest anatomical details with delineation of the internal capsule and WM tracts but no distinction between components of the basal ganglia. Even with the addition of RG, SPGR images quality was not enough to be useful. DWI were granular but of sufficient diagnostic quality.

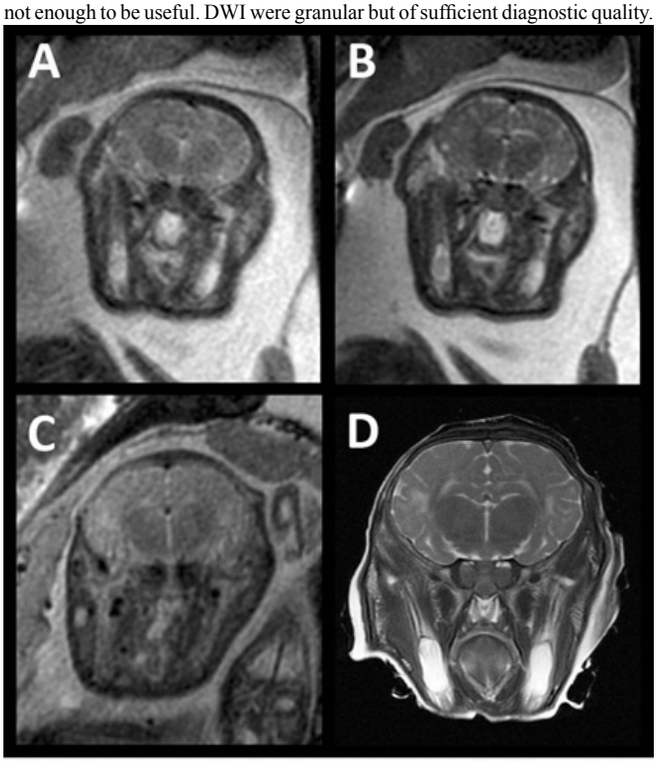


Fig. 1 - Transverse MRI of an *in utero* near-term fetal sheep brain at the level of the diencephalon. **A and B:** T2w FSE images are best suited for visualization of anatomical details. CIR will appear as areas of hyperintensity within cerebral cortex. Addition of RG **(B)** increased conspicuity of structures such as WM tracts and thalamus lobes. STIR without RG **(C)** shows better GM/WM distinction then non-gated T2w FSE **(A)**. **D:** High resolution *ex vivo* T2w FSE.

CONCLUSIONS

By allowing insights into in vivo dynamics of CIR, fetal brain MRI will enhance our understanding of the pathophysiology of evolving cerebral injury in the ovine model and may eventually lead to better diagnostics in human fetal and neonatal MRI.

S-044

Short and Long Term Outcome after Prenatal Diagnosis of Obstructive Megacystis. Esther Passchyn,[1] An Hindryckx,[1] Luc De Catte,[1] Elena Levtchenko,[1] Jan Deprest,[1] Dominique Trouet,[2] Roland Devlieger.[1] [1]*Department of Obstetrics and Pediatric Nephrology, University Hospitals KULeuven, Leuven, Belgium;* [2]*Department of Pediatric Nephrology, University Hospital Antwerp, Antwerp, Belgium.*

Objective

The aim of this retrospective, multicentre cohort study was to evaluate the short and long-term outcomes of children diagnosed with obstructive megacystis in the prenatal period.

Methods

We reviewed the antenatal and pediatric medical records of 132 children with a prenatal diagnosis of megacystis between 2000 and 2011. Prenatal ultrasound and biochemical data, peripartum, neonatal and pediatric data were analysed with descriptive and comparative statistics according to a univariate and multivariate model.

Results

123 children with a prenatal diagnosis of megacystis were included. The average length of follow-up was 5.9 years (range 0-11y). Megacystis was diagnosed in the first trimester of pregnancy in 34/123 cases (27.6%), in the second trimester in 65/123 (52.8%) cases and in the third trimester in 24/123 (19.5%) cases. Chromosomal abnormalities were found in 5 fetuses (4.1%). Termination of pregnancy was performed in 43/123 cases (35%), 8 fetuses died in utero (6.5%), 7 infants died in the early neonatal period (5.7%) and one child died in his first year of life. Fifteen fetuses underwent vesico-amniotic shunting, with a survival of 60% (9/15), 2 survivors had severe renal impairment. Prenatal parameters associated with a lower survival rate were first trimester diagnosis ($p < 0.0001$), oligohydramnios (p: 0.0072), a thick bladderwall (p:0.0012), keyhole sign (p:0.0004), hydronephrosis (p:0.0259) hyperechogenic cortex (p: 0.0023). A low urinary osmolality (p:0.0259), low urinary calcium (p:0.0314) and sodium (p:0.0223) were associated with a higher survival rate. Antenatal hydronefrosis (p:0.0047), hydro-ureters (p:0.0120), the presence of cortical cysts (p0.0487) and oligohydramnios (p:0.0005) were associated with a significantly worse renal function at the age of 2 and 5-8 years. The level of β2-microgloblin in fetal serum was correlated with renal function at 2 and 5 years.

Conclusion

Fetal megacystis associated with lower urinary tract obstruction is a severe condition with a high mortality. In selected cases with good renal function antenatal therapy can be offered to the parents. First trimester diagnosis, oligohydramnios, hyperechogenic renal cortex and high fetal serum β2-microglobulin are associated with poor outcome.

S-045

A Maturational Index for Fetal Brain Development and Its Variation with Fetal State. Hari Eswaran,[1] Srinivasan Vairavan,[1] Rathinaswamy B Govindan,[2] Naim Haddad,[1] Eric Siegel,[1] Curtis L Lowery.[1] [1]*OB/GYN, University of Arkansas for Medical Sciences;* [2]*Fetal and Neontal Medicine, Childrens National Medical Center.*

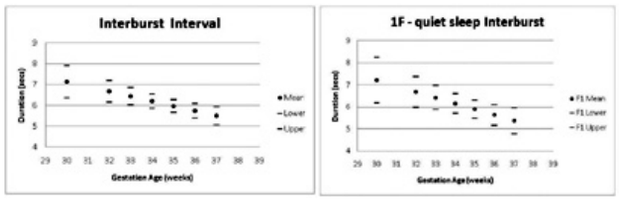
Objective: The goal of the study was to develop a quantitative maturation index for fetal brain development and study its variation with fetal state.

Methods: Using non-invasive magnetoencephalography (MEG) the brain activity was examined in 23 low-risk fetal recordings. The fetal gestational ages (GA) ranged from 28-39 weeks. The discontinuous patterns in spontaneous brain activity are characterized by the occurrence of bursts periods of quiescence. The inter-burst interval (IBI) and the duration of the burst (BD) were explored as possible neurological maturational indices similar to the ones applied in EEG of preterm infants. Fetal discontinuous patterns were scored by two neurophysiologists in a non-overlapping 30-sec window. The BD and IBI were calculated and plotted against gestational age. Further we determined the fetal states of quiet sleep (1F) and active awake (2F) and studied their variation with the burst parameters.

Results: The IBI decreases significantly (Figure1) as the fetus matures which is evident in the small negative correlation across the datasets ($r = -0.14$; p-value <0.01). However, the BD did not show any significant trend. During the quiet sleep state (1F) there is a significant decrease in the IBI durations with GA as indicated by the statistically-significant negative correlation across the datasets ($r = -0.16$, p-value < 0.01). However, the IBI did not show any significant trend in the 2F state. The BD computed during 1F and 2F also did not show any significant trend with GA.

Conclusion: EEG studies on pre-term infants shows that IBI rather than BD is sensitive to brain maturity and there is a decrease in discontinuity and IBI over conception age. Our results show a similar trend along with effect of fetal

state and thus can serve as a normative index for fetal neurological maturation. Prolonged IBI is related to intraventricular hemorrhages and fatal outcomes so we plan to apply this index to high-risk fetal population to study the early warning signs of fetal neurological distress.



S-046

Effects of Acute Hypoxic-Acidemia on Electrocorticogram (ECoG) State Activity in Fetal Sheep: Contribution of Hypoxic Pre-Conditioning (HPC). M Frasch,[1,2] C Duchatellier,[2] H Hamrahi,[1,3] A Bocking,[3] B Richardson.[1]
[1]OBGYN, UWO, London, ON, Canada; [2]OBGYN, U de Montréal, QC, Canada; [3]OBGYN, U of T, Toronto, ON, Canada.

Background: Intermittent hypoxia occurs in ~10% of fetuses and may provide neuroprotection during repetitive exposure to more severe hypoxic-acidemia near-term or during labour. We hypothesized that HPC will improve ECoG state architecture compared to a one-time severe hypoxic insult.

Methods: Near-term fetal sheep were instrumented with vascular catheters, ECoG electrodes and an umbilical cord occluder. The ''Naïve'' group (n=7) received no umbilical cord occlusions (UCO) during Day 1 (D1) to D6 and 4 min UCO each hour x 4 hours on D7 (severe UCO). The ''7d'' group (n=8) received 2 minutes UCO each hour x 4 hours per day from D1 to D6 (to induce HPC) and 4 min UCO each hour x 4 hours on D7. Controls (n=6) received no UCO. On D7, ECoG 95% spectral edge frequency (SEF) was analyzed over 6 hours for relative durations (% time spent) of high voltage/low frequency (HV/LF), low voltage/high frequency (LV/HF) and indeterminate voltage/frequency (IV/F) ECoG states and for fragmentation (number of states per hour) to assess the cumulative hypoxia/asphyxia impact on ECoG state architecture. Significant differences are reported for $p<0.05$ as mean±SEM.

Results: Moderate UCO over 6 days resulted in intermittent hypoxia without worsening acidemia. On D7, severe UCO resulted in worsening metabolic acidemia in both UCO groups. On D7, the SEF ECoG state cut-off criteria were similar for all groups at 20±1 Hz for LV/HF and 14±1 Hz for HV/LF groups. We found no differences in ECoG state characteristics between the naïve and 7d UCO groups. LV/HF and fragmentation decreased in naïve group to 19±2% and 1.7±0.1 and in 7d UCO group to 24±3% and 1.8±0.2, vs. Control (46±5% and 3.1±0.3). In contrast, the HV/LF was unchanged at ~28±3%. However, HV/LF fragmentation decreased to 1.8±0.4 for both UCO groups vs. Control (3.2±0.3). Concomitantly, the relative IV/F duration increased to an average of 50±6% in both UCO groups vs. Control (20±3%).

Conclusions: Acute repetitive intermittent hypoxia with metabolic acidemia resulted in ECoG state re-modeling without further modulation by a weaker preceding chronic intermittent hypoxic stimulus. Decreasing %time spent in the energy demanding LV/HF and increasing IV/F are in line with previous findings. Repetitive UCO also led to a parallel decrease of ECoG state fragmentation suggesting adaptations of state generation.

S-047

Effect of Fetal Hypoxia on AT1R and AT2R Expression and Kidney Development. Pablo J Gonzalez-Rodriguez, Qin Xue, Shirley Hu, Lubo Zhang. *Basic Science, Loma Linda Univesity School of Medicine, Loma Linda, CA, USA.*

Introduction: Environmental insult during critical periods of development can reprogram normal physiological responses and give rise to metabolic and cardiovascular disorders later in life. Hypoxia is one of the most common environmental insults during gestational process. Our hypothesis is that prenatal hypoxia may affect the kidney development in fetal and offspring rats by altering the expression of angiotensin II receptor type 1 (AT1R) and type 2 (AT2R).

Methods: Time-dated pregnant rats were divided between normoxic (N) and hypoxic (H) (10.5% O2 from day 15 to 21 of gestation) groups. Nephrectomy was done in 21-day fetuses (E21), 7-day neonates (P7) and 3 months old (3m) males and female offspring. Protein abundance of AT1R and AT2R was determined by Western blot.

Results: Hypoxic treatment significantly decreased body and kidney weight in E21 and P7. The kidney to body ratio was decreased in E21 but increased in P7 in hypoxic rats compared with normoxic animals. In adult offspring there were no significant differences in body and kidney weight. No significant differences were found in kidney AT1R density at the developmental stages of E21 and P7 due to fetal hypoxia, however, in adults the expression of AT1R decreased significantly in males and in females. In contrast, kidney AT2R density was not affected by fetal hypoxia throughout the developmental stages studied. There was a small but significant decrease in kidney glomerular numbers in P7 of hypoxic rats (11887 ± 472), as compared with the normoxic pups (13629 ± 601) ($p < 0.05$). This difference between the groups increased during the postnatal development at 3m adults more in females (N: 30352 ± 2016 vs. H: 14762 ± 651, $p < 0.001$) than that in males (N: 24002 ± 503 vs. H: 17833 ± 1935, $p < 0.001$).

Conclusion: Fetal hypoxia adversely affects kidney development by reducing the number of nephrons during early developmental stage, and this effect is progressively worsen into the adulthood with females affected more than males. This hypoxia-mediated, postnatal development-dependent reduction of nephron numbers is associated with decreases in AT1R density in the kidney. These results suggest that fetal hypoxia causes programming of aberrant kidney development and accelerates the aging process of the kidney, which may contribute to an increased risk of cardiovascular disease, in particular hypertension. (Supported in part by NIH grants HL83966 and 5 P20 MD001632)

S-048

Glucocorticoids (GCs) Increase Multidrug Resistance in the Developing Blood-Brain-Barrier (BBB). Majid Iqbal,[1] William Gibb,[2] Stephen G Matthews.[1] *[1]Physiology, Obstetrics & Gynecology and Medicine, University of Toronto, Toronto, ON, Canada; [2]Obstetrics & Gynecology and Cellular & Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.*

Objective: P-glycoprotein (P-gp) protects the fetal brain from a wide range of xenobiotics and regulates the passage of endogenous compounds. P-gp levels in the early fetal brain are low, but dramatically increase near term. This increase is concurrent with the late gestation rise in endogenous GCs. GCs potently stimulate P-gp, and fetal brain endothelial cells (BECs) become more sensitive to GCs in late gestation. However, whether the endogenous GC surge is responsible for the rise in BBB P-gp is unknown. Using BEC cultures derived from fetuses exposed to synthetic GCs (sGCs) in utero, we investigated the regulatory effects of sGCs on P-gp function and BEC responsiveness to GCs. We hypothesized that prenatal sGC exposure will enhance P-gp function – mimicking the endogenous GC surge.

Methods: Brain microvessels (BMVs) and BECs were isolated from gestational day (GD) 50 fetuses exposed in utero to dexamethasone (sGC; 1 mg/kg), or vehicle (VEH) on GD48/49. Confluent BECs were treated with 10∧-8-10∧-5 M of cortisol, dexamethasone or aldosterone for 2-24h, and P-gp function was assessed (1 μM calcein-AM). P-gp protein was measured in BMVs.

Results: Treatment of VEH-exposed GD50 BECs with cortisol resulted in a trend of increased P-gp activity, but this was not significant ($P>0.05$). However, GD50 BECs derived from sGC-exposed fetuses displayed a significant dose-dependent increase in P-gp function when treated with cortisol (~40%; $P<0.01$). Treatment of BECs from both VEH and sGC-exposed fetuses with dexamethasone resulted in increased P-gp function (~40%; $P<0.01$), while aldosterone treatment had no effect ($P>0.05$). Prenatal sGC exposure increased in BMV P-gp protein, 2-fold ($P<0.05$).

Conclusions: GCs are centrally involved in the regulation of multidrug resistance at the developing BBB. Exposure to sGCs prior to the endogenous GC surge matures BEC response to GCs – so that the response mimics that seen in near-term BECs (experienced endogenous GC surge). Mothers at risk of preterm labor are administered sGC to mature the fetal lungs. Our data suggests that this treatment prematurely increases fetal BBB multidrug resistance expression, potentially altering fetal brain protection against xenobiotics, but also the passage of endogenous compounds important for development.

Funded by: Canadian Institutes for Health Research.

S-049

Maternal Nutrient Restriction (MNR) from 30 – 165 days of Gestation (G) Decreased Neurotrophin (NT)-3 Expression, but Not Its Tyrosine Kinase (Trk)C Receptor in Fetal Baboon Frontal Cortex (FC). Manabu Kemmochi,[1] Michiyo Nakamura,[1] Cun Li,[2] Peter W Nathanielsz,[2] Thomas J McDonald.[2] *[1]Dept. of Pediatrics, Social Insurance Sagamino Hospital, Sagamihara City, Kanagawa, Japan; [2]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Background: In development, NTs ensure appropriate target innervation density and regulate axon and dendrite growth and pruning and expression of critical proteins, e.g., ion channels and neuro-transmitters and NT importance continues throughout postnatal life. Our MNR in baboon pregnancy model shows altered fetal brain molecular phenotype and modified postnatal cognition (Rodriguez J, Reproductive Sciences in Press). We investigated effects of MNR on NT-3 and TrkC peptide expression to determine if they could contribute to the behavioral phenotype.

Methods: Adult female baboons were fed *ad lib* (CTR) or 70% global CTR diet (MNR) from 0.16 to 0.9 G (CTR, n=7; MNR, n = 6) with fetuses taken at C-section under general anesthesia. Peptide expression for NT3 and TrkC was determined in fetal frontal cortex (FC) by immunohistochemistry (IHC) and western blot (WB). Statistical analysis - Student's t-test with α level set at 0.05, data as mean ± SEM; CTR data presented first.

Results: NT-3 peptide expression was decreased($p< 0.05$) in FC of MNR fetuses compared to CTR by IHC and WB (Fig. 1), but no difference ($p > 0.05$) was seen in TrkC receptor by IHC (1.3 ± 0.34 vs. 1.04 ± 0.27 %) or WB (0.32 ± 0.06 ± 0.25 ± 0.05 DU).

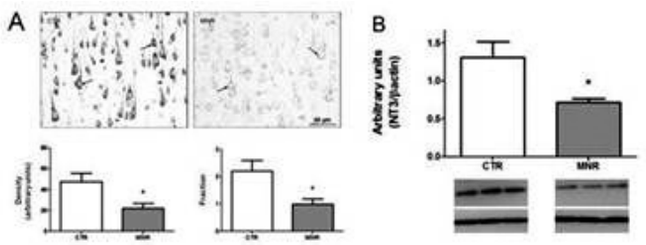


Figure 1. Micrographs and summaries for NT-3 peptide expression as measured by IHC (A) for Density (arbitrary units) and Fraction (% area immunostained) and by WB (B) in frontal cortex of fetuses from mothers *ad lib* fed (open bars) CTR or fed 70% CTR diet (MNR; closed bars) from 30 – 165 days of gestation. Data as mean ± SEM, * $p< 0.05$.

Conclusion: Given the importance of NT-3 in neurodevelopment, the observed reduction in NT-3 peptide expression may play a role in the learning and behavioral deficits we have shown in our model and have been described for children growth restricted as fetuses (J Pediatr 125: 426).

S-050

Maternal Nutrient Restriction (MNR) Effects on Glial Fibrillary Acidic Protein (GFAP), the Main Intermediate Filament Protein in Astrocytes. Cun Li, Mark J Nijland, Peter W Nathanielsz, Thomas J McDonald. *OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Introduction: GFAP, the main intermediate filament in astrocytes, once thought to have a purely structural role, has now been shown to be involved in many crucial astrocyte and neuronal processes including: astrocyte motility and migration, proliferation, chaperone-mediated autophagy, synaptic plasticity, glutamate transport and glutamine synthesis, neurite outgrowth, blood brain barrier functioning, myelination and injury protection. Our FC gene arrays indicate that MNR group baboon fetuses were hypometabolic, i.e., mRNA for 30 metabolic pathways significantly decreased ($Z > 1.99$), which included significantly ($p = 0.02$) decreased GFAP mRNA in frontal cortex (FC) of MNR fetuses compared to fetuses of *ad lib* fed mothers (CTR). Based on our gene discovery studies, we hypothesized that GFAP peptide expression also would be decreased in this nutrient deprivation paradigm.

Methods: Pregnant baboons were fed *ad lib* (CTR) or 70% CTR global diet (MNR) from 0.16 - 0.9 G (term 184 d) with tissue collection under general anesthesia. FC GFAP peptide expression was determined by semi-quantitative immunohistochemistry and quantified by image analysis (ImageJ, NIH) for Fraction (% area immunostained) and Density (arbitrary units). Statistical analysis was by Student's t-test, data are expressed as mean SEM and α level set at 0.05.

Results: GFAP Fraction and Density in FC were both significantly ($p< 0.05$) decreased in MNR group fetuses in comparison to CTR (see Figure 1).

Conclusion: Our results suggest that the observed loss of GFAP, with its wide-ranging functions mentioned above, plays a key role in the observed FC hypometabolism.

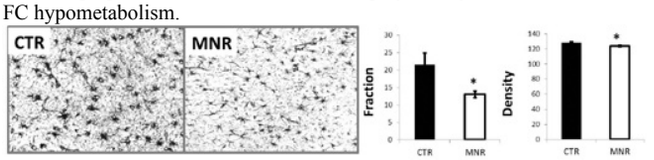


Figure 1. GFAP peptide expression micrographs and summary graphs as determined by immunohistochemistry with counting program, in FC astrocytes of fetuses from mothers *ad lib* fed (closed bars) or fed 70% CTR diet (open bars) from 0.16 - 0.9 G. Data expressed as mean SEM, * $p< 0.05$.

S-051

Decreased Fetal Amino Acid (AA) Availability Upregulates the Neutral AA Transporter SNAT2 in Fetal Baboon Frontal Cortical (FC) Neurons and Glia Indicating Fetal Brain Compensation. Cun Li,[1] Claudia Bautista,[2] Laura A Cox,[3] Peter W Nathanielsz,[1] Thomas J McDonald.[1] *[1]Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA; [2]Instituto Nacional de Ciencias Medicas y Nutricion, Mexico City, Mexico; [3]Genetics, Texas Biomedical Research Institute, San Antonio, TX, USA.*

Introduction: Maternal nutrient restriction (MNR) in baboon pregnancy decreases placental size and lowers placental expression of SNAT2 thus decreasing term fetal plasma methionine, a key one carbon cycle (1-CC) AA for DNA synthesis and epigenetic gene regulation. In embryonic rats, FC immunohistochemistry (IHC) localized SNAT2 to radial glia and to dendrites in late gestation (G; Int J Dev Neurosci EPub 2011). Because of the essential nature of the 1-CC we hypothesized that decreased methionine availability results in a compensatory increase in FC SNAT2 expression.

Methods: Pregnant baboons were fed *ad lib* (CTR) or 70% global CTR diet (MNR) from 0.16 - 0.9 G (term ~ 184 days) with tissue collection under general anesthesia. FC SNAT2 protein was quantified by IHC for Fraction (% area immunostained) and Density (arbitrary units) and mRNA by Illumina array on flash frozen FC. Statistical analysis was by Student's t-test, data are expressed as mean ± SEM with α level set at 0.05 and CTR data presented first.

Results: SNAT2 peptide expression was significantly ($p< 0.05$) increased in neurons (Fig. 1) and in glia for Fraction (CTR 2.5 ± 0.6 vs. MNR 6.0 ± 1.0%, $p< 0.01$), but not Density (CTR 77.8 ± 1.3 vs. MNR 79.7 ± 1.1, $p> 0.05$).

Conclusions: Since SNAT2 mRNA was unchanged in MNR it is likely that the protein increase is due to post-translational, potentially epigenetic regulation. The increased SNAT2 protein expression indicates FC attempts to maximize AA capture when availability is reduced indicating the importance of adequate AA supply in successful development.

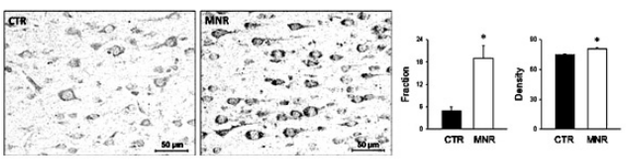


Figure 1. SNAT2 protein summary determined by IHC in FC neurons of fetuses from mothers *ad lib* fed (closed bars) or nutrient restricted (open bars; fed 70% CTR diet) from 0.16 – 0.9 G. Data are mean ± SEM, * $p< 0.01$.

S-052

Contrasting Effects of Maternal Nutrient Restriction (MNR) on Fetal Baboon Frontal Cortex (FC) Glycogen Storage at 0.5 Vs. 0.9 of Gestation (G). Cun Li, Mark J Nijland, Peter W Nathanielsz, Thomas J McDonald. *OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.*

Introduction: Astrocytic glycogen, the sole brain carbohydrate reserve, is an important energy reservoir for astrocytes and neurons, but is not found in postnatal neurons. A recent study of 10 day-old nutrient reduced rat pups, a similar developmental stage as our late G fetuses, showed increased glycogen expression in FC astrocytes (J neurochem 112:123). Thus, we hypothesized that glycogen would be increased at 0.9G in FC astrocytes of our MNR group fetal baboons compared to controls, but not in 0.5G FC.

Methods: Pregnant baboons were fed *ad lib* (CTR) or 70% CTR global diet (MNR) from 0.16 - 0.9G (term 184 d) with tissue collection under general

anesthesia. Glycogen expression was shown by the periodic acid-Schiff (PAS) stain and quantified by image analysis (Image J) for Fraction (% area stained) and Density (arbitrary units). Statistical analysis: Student's t-test, data as mean SEM, CTR data first, α level set at 0.05.

Results: At 0.9G, glycogen expression was extensive in FC astrocytes, but not localized in neurons (unstained ovals Fig. 1, 0.9G MNR photo) with no difference between CTR and MNR (Fraction: 56.8 ± 5.7 vs. 67.3 ± 6.1 , p > 0.05 and Density: 27.6 ± 0.49 vs. 28.8 ± 0.86 DU, p > 0.05). However, at 0.5G, glycogen was increased (p< 0.05) in MNR FC and was localized in both astrocytes and neurons (Fig. 1).

Conclusions: The lack of a glycogen increase with MNR in astrocytes near term does not fit with data from the only other fetal paper we know of (J neurochem 112:123). In addition, little if any information is available on FC glycogen expression in the first half of G. Thus we conclude that more study is needed in this potentially important area of research with implications for undernutrition and fetal brain sparing.

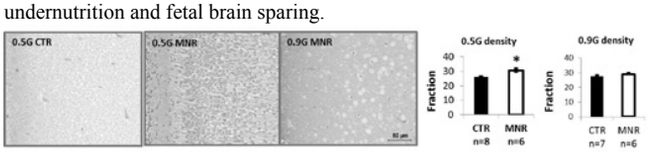


Figure 1. FC glycogen micrographs and summaries determined by PAS stain in astrocytes and neurons of fetuses at 0.5 G (left & center) and astrocytes at 0.9 G (right), from mothers fed *ad lib* (CTR) or 70% CTR diet (MNR) from 0.16 0.9 G. Data are mean SEM, * p< 0.05.

S-053

Maternal Nutrient Restriction (MNR): Effects on Mitochondrial Ribosomal Protein Subunit (MRPS) Expression in Late Gestation (G) Fetal Baboon Frontal Cortex (FC) and Heart. Lynn Xie, Cun Li, Mark J Nijland, Peter W Nathanielsz, Thomas J McDonald. *OB/GYN, Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: Most energy required for development, growth and differentiation is supplied by the mitochondrial oxidative phosphorylation end product ATP. Interestingly, nutrient restriction during fetal life is involved in multiple metabolic deficiencies that are exacerbated with post-natal aging. In discovery studies, our FC gene arrays indicated that fetal baboon mRNAs for 9 large and 5 small MRPSs were significantly (p< 0.01) decreased and also showed this same group of baboon fetuses to be hypometabolic, i.e., 30 metabolic pathways significantly decreased (Z > 1.99) in FC of MNR group fetal baboons. We hypothesized that MRPS peptide expression would also be reduced in FC and heart of late gestation MNR group fetuses.

Methods: Adult female baboons were fed *ad lib* (CTR) or 70% CTR global diet (MNR) from 0.16 - 0.9 G (term ~ 184 d) with fetuses taken at c-section under general anesthesia. Peptide expression in FC and heart was determined by semi-quantitative immunohistochemistry (IHC) with primary antibodies to MRPSs L23 and S14 and quantified by image analysis (ImageJ, NIH) for Fraction (% area immunostained) and Density (arbitrary units) and mRNA of flash frozen FC by Illumina arrays. Statistical analysis: Student's t-test with data expressed as mean ± SEM, CTR data presented first, α level set at 0.05.

Results: MRPSs L23 and S14 were significantly (p< 0.05) or close to significantly (0.049 < p < 0.1) decreased for all cases for both FC and heart (see Table 1).

Table 1. MRPS L23 and S14 peptide expression in heart and FC of fetuses from baboon mothers fed *ad lib* (CTR) or 70% CTR diet (MNR) from 0.16-0.9 gestation

MRPS	Fraction		Density	
	CTR	MNR	CTR	MNR
Heart L23	38.89±3.6	29.02±2.7*	76.0±2.3	76.0±2.3#
Heart S14	52.97±4.6	44.41±2.4*	78.69±1.8	74.29±0.8*
FC L23	5.76±0.35	1.3±0.40**	59.9±1.7	55.6±0.78*
FCS14	6.27±0.96	1.69±0.54*	59.66±1.5	59.17±0.96^

** p< 0.01, * p< 0.05, # p = 0.05, ^ 0.05 > p < 0.1

Conclusion: Our results suggest that aerobic glycolysis is reduced in fetal FC and heart with MNR in late gestation, which would contribute to the observed fetal hypometabolism used by the MNR group fetuses for survival.

S-054

Maternal Nutrient Restriction (MNR) in the Baboon Impairs Neuronal Migration to the Frontal Cortex (FC) Accompanied by Upregulation of Glutaminergic NMDA Receptors (NMDAR) at 0.5 Gestation (G) and Down-Regulation by 0.9G: Potential Role of Circulating Amino Acids (AA) Known To Regulate NMDAR. Thomas J McDonald,[1] I Antonow-Schlorke,[2] Matthias Schwab,[2] Cun Li,[1] Mark J Nijland,[1] Guoyao Wu,[3] Peter W Nathanielsz.[1] *[1]OB/GYN, Center for Pregnancy and Newborn Research, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA; [2]Hans Berger Department of Neurology, Friedrich Schiller University, Jena, Germany; [3]Texas A&M University, College Station, TX, USA.*

NMDARs regulate neuronal growth and act as chemoattractants stimulating migration and synaptic development (J. Neurosci. 1999, 19:4449). We have demonstrated a failure of neuronal migration from the subventricular zone to the FC in fetuses of MNR baboons (Proc Nat Acad Sci. 108:3011). MNR also reduces availability of key fetal AA such as glycine (GLY) that act as NMDAR co-activators.

HYPOTHESIS: MNR decreases FC NMDAR resulting in impaired migration of immature nerve cells to the fetal baboon FC.

METHODS: Pregnant baboons were fed either control diet (C) or a MNR diet (70% of diet consumed by controls, n=8) from 0.16 gestation (G) to 0.5G (n=8; male fetuses n=3, female n=5); or MNR (n=6; male n=3,female n=3) or from 0.15 G to 0.9G ; (C n=7; male n=3, female n=4) or MNR n=6; ; male n=3,female n=3). Fetuses were removed at CSection under general anesthesia and FC quantified for the following proteins by immunohistochenistry (IHC): NMDAR 1, 2A and 2B, Neun for mature neurons, synaptohysin and golgi staining. Plasma AA measured by HPLC.

RESULTS:

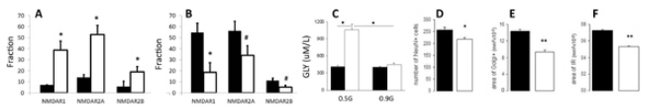


Fig 1. 1A) At 0.5G MNR increased all three NMDAR which B) were decreased by MNR at 0.9G. 1C) fetal plasma glycine was elevated at 0.5G vs C but had fallen to C levels at 0.9G; D) FC showed delayed cell migration and structural development as D) mature neurons E) golgi staining and F) synaptophysin immunoreactivity all decreased. There were no differences between female and male.

CONCLUSIONS: The biphasic effect on NMDAR has major potential consequences for brain development with potential for long term effects on cognitive and other behaviors including adddiction.

S-055

ERK 1/2 Plays a Role in eNOS Phosphorylation and Cortisol Biosynthesis in the Ovine Fetal Adrenal. Elizabeth Newby,[1] Christina Hess,[1] Kanchan Kaushal,[1] Dean Myers,[2] Charles Ducsay.[1] *[1]Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA; [2]Department of Ob/Gyn, University of Oklahoma Health Sciences Center, Olkahoma City, OK.*

Background: We have previously shown that nitric oxide (NO) plays a role in regulation of cortisol synthesis in the ovine fetal adrenal and that the MEK/ERK 1/2 inhibitor UO126 (UO) reduces cortisol production. Although the role of ERK 1/2 in nitric oxide synthase (NOS) activation (phosphorylation) is controversial, it appears that it does play a role in regulating NOS activity. The present study was designed to determine the effect of ACTH and UO on eNOS phosphorylation and cortisol production in fetal adrenal cortical cells (FACs).

Materials and Methods: Adrenal glands were collected from near term (139-141 days gestational age, term =146 days) ovine fetuses (n=7). The dispersed FACs were untreated (control), pretreated with 10μM UO, treated with ACTH (100pM) alone, or pretreated with UO and then challenged with ACTH. Samples were collected at 0, 10, 20, and 60 min for measurement of cortisol and protein expression of eNOS and phosphorylated eNOS (p-eNOS, SER1177/79) by Western blot (relative optical density, ROD).

Results: A significant increase in cortisol secretion was observed following ACTH stimulation that was blocked by UO pretreatment (Table 1). UO treatment also resulted in a decrease in p-eNOS protein during the course of the study that was significant at 60 min, while eNOS expression did not differ among treatment groups.

Table 1. Effect of UO and ACTH on Cortisol and p-eNOS

	Baseline (0 min)	Control (60 min)	UO (60 min)	ACTH (60 min)	UO+ACTH (60 min)
Cortisol (ng/7.5x10^{5}cells	6.50±1.21	6.21±1.31	5.96±1.00	13.48±2.66*	7.62±1.24
p-eNOS (ROD)	2.96±0.40	2.17±0.24	1.41±0.25*	1.91±0.45	1.16±0.28*

*p<0.05 compared with baseline (0 min)

Saturday

Conclusions: ERK 1/2 inhibition significantly reduced phosphorylation of eNOS at SER 1177/1179 and resulted in inhibition of ACTH-stimulated cortisol secretion by FACs. Together, these data suggest that the extracellular signal regulated kinases play a key role in cortisol biosynthesis and may do so in part through regulation of eNOS activation. (supported by NIH grant 31226)

S-056

Gender-Specific Effects of 30% Global Maternal Nutrient Restriction (MNR) on Non-Human Primate Renal Mitochondrial Transcripts at 0.9 Gestation (G). Susana P Pereira,[1,2] Paulo J Oliveira,[1] Laura A Cox,[3] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Center for Neurosciences and Cell Biology, Univ. Coimbra, Coimbra, Portugal; [2]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA; [3]Department of Genetics, TX Biomed, San Antonio, TX, USA.*

Epidemiological studies have established that early-life malnutrition generates structural changes in the kidneys that predispose offspring to renal dysfunction. Recent examination of renal autopsies of adults born with a low birth weight revealed substantial variation in renal composition indicative of an innate capacity of the kidney to overcome a hostile fetal environment.

Due to the role of mitochondrial bioenergetics in energy metabolism and kidney function, the objective of this study was to analyze the impact of MNR on fetal renal mitochondrial transcript expression.

Female baboons were fed normal chow (C) or 70% of C (MNR). A cross-section of fetal kidney was obtained via c-section at 165 days (0.9 G), and stored at -80°C, and analyzed using qRT-PCR array. Data was analyzed using GraphPad Prism v5 with significance set at $p < 0.05$.

MNR did not affect fetal kidney:body weight ratio. Despite lack of morphological effect, the plasma amino acid profile was significantly altered in MNR animals with lower levels of both essential and non-essential amino acids and plasma cortisol was increased. Differential expression of mRNA related to mitochondrial metabolite transport and dynamics was found in females (F) vs males (Down: TIMM17A, SLC25A17, OPA1; Up: COX6A1, IMMP1L and SFN). MNR-related alterations in expression were more evident in F, with 16 transcripts significantly altered, 14 down-regulated (NDUFV3, NDUFS5, COX6A2, COX7B, ATP5J, ATP12A as well as several solutes carriers) and 2 up-regulated (CDKN2A and SLC25A15). A total of 9 transcripts were different in M, with 7 down-regulated (specifically coding for complex I and IV and TIM/TOM complex subunits) and 2 up-regulated (SFN and TIMM9).

Renal transcripts that code for key players of mitochondrial energy metabolism are susceptible to the effects of MNR in a sex-dependent manner. We speculate that these differences can lead to decreased mitochondrial efficiency that, in turn, impacts normal kidney development and susceptibility to mitochondrial-mediated renal pathologies in the adult life.

Supported by NIH PO1 HD021350 (MJN, PWN) and Portuguese FCT SFRH/BD/64247/2009 (SPP).

S-057

The Impact of Fetal Growth Restriction on Brain Development in the Guinea Pig at Term. Karolina Piorkowska,[1,2] Robert Hammond,[3] Brad Matushewski,[2] Bryan Richardson.[1,2,4] *[1]Physiology, University of Western Ontario; [2]Obstetrics and Gynaecology, University of Western Ontario; [3]Pathology, University of Western Ontario; [4]Paediatrics, Children Health Research Institute.*

Fetal growth restriction (FGR) resulting from placental insufficiency has an underlying risk for subsequent cognitive disorder indicating aberrant brain development. Altered substrate metabolism and activity-dependent remodelling may affect synaptogenesis and myelination of neuronal connections in areas essential for learning and memory. Synaptophysin (SYN), a presynaptic vesicle membrane protein, and synaptopodin (SYNPO), a postsynaptic dendritic spine protein, are essential for synapse formation while myelin basic protein (MBP) which is abundant in the myelin sheath surrounding neurons, is essential for stabilization of neuronal communication. This study examines changes in the expression of these synaptic and myelin proteins with FGR in the guinea pig brain at term. Chronic placental insufficiency was generated by selective cauterization or unilateral ligation of the uterine artery in pregnant guinea pigs at mid-gestation. At ~65 days gestation (term), fetal brains were processed for immunohistologic examination of SYN, SYNPO, and MBP in the hippocampal CA1,CA3 and dentate gyrus (DG) areas, hippocampal efferents; the fimbria and fornix, parasagittal grey and white matter, the corpus callosum (CC), periventricular white matter (PVW) and the optic tract (OT). Fetal pups were selected for analysis based on birth weight and brain to liver weight ratio and assigned to appropriate (AGA), symmetrical FGR (sFGR) or asymmetrical FGR (aFGR) groupings. Compared to AGA animals, sFGR animals showed a 40-55% decrease in SYN in the hippocampal CA1,CA3 and DG areas and in SYNPO in the CA1 and CA3 areas (all $p<0.01$). The aFGR animals had 26% decrease in SYN in the CA1 and CA3 areas (both $p<0.05$) and an 18% decrease in the DG (ns). SYNPO was unchanged in aFGR animals in the hippocampal regions and in the DG of sFGR animals. There was no difference in SYN in the parasagittal grey matter or CC. There was a 21%-50% decrease in MBP in sFGR in the hippocampal efferents, CC, PVW and parasagittal white matter (all $p<0.02$) and 15%-59% decrease in aFGR animals (all $p<0.05$, but fimbria ns). There was no difference in MBP in the OT. The decrease in SYN, SYNPO, and MBP expression indicates fewer mature neuronal connections with FGR suggesting an etiology for later neurologic sequellae.

S-058

Fetal Cholinergic Anti-Inflammatory Pathway (CAP) Modulates Microglial (MG) High-Mobility Group Box 1 (HMGB1) Protein Release during Cerebral Inflammatory Response. D Siontas,[1] K Nygard,[2] G Ponce,[2] M Frasch.[1] *[1]OBGYN, U de Montréal, QC, Canada; [2]Integrated Microscopy@ Biotron, UWO, London, ON, Canada.*

BACKGROUND: In near-term fetal sheep umbilical cord occlusions (UCO) leading to severe acidemia result in vagal activation and MG activation in white matter (WM). In adults with post-ischemic injury, microglial HMGB1 translocates from nucleus to cytosol and extracellular space (ESC). Via CAP, increased vagal activity inhibits release of inflammatory cytokines such as HMGB1. RMSSD, a measure of fetal heart rate variability (fHRV), reflects vagal activation and CAP activity. We hypothesized that UCO will lead to HMGB1 translocation and that due to the α7 nicotinic acetylcholine receptor (α7 nAChR) expressed in MG, the degree of vagal activation and HMGB1 translocation will correlate.

METHODS: Near-term fetal sheep were instrumented with vascular catheters, ECG, and umbilical cord occluders. The UCO group (n=9) received a series of UCO over 4h until arterial pH decreased to <7.00 and, along with 5 controls, were euthanized at 24h of recovery. Grey matter layers 1-3 and 4-6 (GM13, GM46), WM, thalamus and hippocampus (dentate gyrus, DG) were quantified for HMGB1 in Iba1+ and α7 nAChR+ MG. Morphology discriminated active and quiescent microglia (aMG, qMG). RMSSD was derived from the ECG.

RESULTS: In contrast to neurons and astrocytes, in aMG and qMG of all regions except GM46, nuclear intensity per area (IA) was greater than cytosolic IA in both groups. The UCO group showed lower nuclear HMGB1 IA in aMG of GM46 and DG and lower cytosolic HMGB1 IA in qMG in GM46 than controls. In WM, aMG and qMG of the UCO group showed lower nuclear HMGB1 IA than controls. α7 nAChR IA in aMG of DG was higher than in qMG in the UCO group (all $p<0.05$). In parallel, RMSSD at pH nadir correlated with α7 nAChR IA in aMG of WM ($R=0.83$, $p=0.06$) and RMSSD at 1h of recovery correlated with cytosolic HMGB1 IA in aMG of thalamus ($R=-0.94$, $p=0.02$).

COMMENTS: We observed pronounced, region-specific MG α7 nAChR expression and HMGB1 translocation. These results together with general MG activation in WM reported earlier suggest HMGB1 release into ESC. We had reported that fetuses with higher RMSSD during acidemia showed lower WM MG activation. Here we show this may be mediated via α7 nAChR in the WM aMG. A CAP-mediated effect of afferent vagal activity on MG HMGB1 release is supported by negative correlation between MG thalamic cytosolic HMGB1 and vagal activation after pH nadir.

S-059

Expression of StAR and Key Genes Regulating Cortisol Biosynthesis in near Term Ovine Fetal Adrenal Cells: Effects of Long Term Hypoxia. Vladimir E Vargas,[1] Dean A Myers,[2] Kanchan M Kaushal,[1] Charles A Ducsay.[1] *[1]Ctr. Perinatal Biol., Loma Linda Univ., Loma Linda, CA, USA; [2]Ob/Gyn, Oklahoma Univ. HSC, Oklahoma City, OK, USA.*

Background: We have previously demonstrated decreased expression of key genes regulating cortisol biosynthesis in the long-term hypoxic (LTH) sheep fetal adrenal glands compared to normoxic control. We have also shown that inhibition of the extracellular signal regulated kinases (ERKs) with UO126 severely limited ACTH-induced cortisol production in ovine fetal adrenocortical cells (FACs), suggesting a role for ERKs in cortisol synthesis. In this study we wanted to study if the observed decrease in LTH CYP11A1 and CYP17 in adrenal glands was maintained in vitro FACs, and if ACTH alone and/or UO126 treatment had any effects on the expression of StAR, CYP11A1, and CYP17 in control vs. LTH FACs.

Methods: Pregnant ewes were maintained at high altitude (3,820m) from ~ 40 days of gestation (dG). At 138-141 dG, fetal adrenal glands were collected

from LTH (n=5) and age-matched normoxic controls (n=6). Dispersed FACs (2.5x100,000 cells/well; in duplicate) were challenged with ACTH (10^{-8}M) with or without UO126 for 18h. mRNA for key enzymes (CYP11A1, CYP17) and StAR was quantified by qRT-PCR. Data are expressed in fg mRNA/12.5 ng cDNA±SEM.

Results: StAR mRNA was decreased in LTH vs. control under basal conditions, following ACTH or ACTH+UO126 ($p<0.05$). UO126 alone had no effect on any mRNA in either group. ACTH increased CYP11A1 and CYP17 in control but not LTH. U0126 prevented the increase in CYP11A1 and CYP17 in control FACs. Basal CYP11A1 and CYP17 were not different between LTH and control FACs.

StAR mRNA	Basal	UO126	ACTH	ACTH + UO126
Control	3.11±0.54	0.78±0.21	5.60±1.63	2.65±0.60
LTH	0.87±0.20[b]	0.21±0.08	1.81±0.27[b]	1.22±0.24[b]

CYP11A1 mRNA	Basal	UO126	ACTH	ACTH + UO126
Control	7.60±1.01	2.84±0.89	31.77±5.84[a]	19.52±3.72
LTH	6.95±1.40	1.81±0.32	18.90±3.28	10.49±1.97
CYP17 mRNA	Basal	UO126	ACTH	ACTH + UO126
Control	0.04±0.01	0.07±0.03	0.44±0.10[a]	0.25±0.07
LTH	0.05±0.02	0.04±0.01	0.23±0.08	0.18±0.07

[a] vs. Basal($p<0.05$); [b] LTH vs. Control ($p<0.05$)

Conclusions: ACTH increased CYP11A1 and CYP17 only in control FACs consistent with our previous observation of slower maturation of the adrenal cortex in response to LTH. U1026 attenuated the ACTH response indicative of a role for ERK in CYP11A1 and CYP17 expression. ACTH may require additional factors in FACs to fully regulate StAR expression. (Grants HD31226 and P20-MD001632)

S-060

Expression of 3-Hydroxy-3-Methylglutaryl-Co-enzyme A Reductase (HMG-CoAR), Hormone Sensitive Lipase (HSL) and the Low Density Lipoprotein Receptor (LDLR) Are Increased in the Adrenal Cortex of the Late Gestation Long Term Hypoxic (LTH) Sheep Fetus. VE Vargas,[1] CA Ducsay,[2] KM Kaushal,[2] KE Hyatt,[1] K Hanson,[1] DA Myers.[1] *[1]Ob/Gyn, Oklahoma Univ. HSC, Oklahoma City, OK; [2]Ctr. Perinatal Biol., Loma Linda Univ., Loma Linda, CA.*

BACKGROUND: The LTH ovine fetus exhibits a normal ontogenic increase in basal plasma cortisol concentrations despite elevated plasma ACTH concentrations. In response to a secondary stressor in vivo, or ACTH in vitro, cortisol production is higher in the LTH compared to control, normoxic fetuses. Paradoxically, expression of key rate limiting enzymes for cortisol synthesis (CYP17, CYP11A1) and the mitochondrial cholesterol transport protein, StAR is lower in the LTH adrenal cortex. In the late gestation ovine fetal adrenal cortex, cholesterol is derived from plasma LDL as well as de novo synthesis. However, the fetal adrenal cortex has limited capacity for both cholesterol uptake (LDLR) or synthesis. We hypothesized that either uptake of LDL via LDLR expression, liberation of cholesterol esters via HSL or de novo synthesis via HMG-CoAR are enhanced in the LTH fetal adrenal cortex providing greater cholesterol for cortisol synthesis.

MATERIALS AND METHODS: Pregnant ewes were maintained at high altitude (3,820 m) for ~100 days. At 138-141 dG, adrenal glands were collected from LTH and age-matched normoxic control fetuses. Quantitative real time PCR (qRT-PCR) was used to measure LDLR, HMG-CoAR and HSL (fg mRNA per 50 ng RNA). Cyclophilin was used as a housekeeping gene. Data is presented as mean±SEM.

RESULTS: HSL (LTH: 0.142 ± 0.019 vs. CONT: 0.135 ± 0.02) was not different between LTH and control fetal adrenal cortices. HMG-CoAR (LTH: 13.44 ± 0.82 vs CONT: 7.72 ±1.52) and the LDLR (LTH: 11.22 ± 0.79 vs. CONT: 4.12 ± 0.99) were significantly ($p<0.05$) elevated in the adrenal cortex of LTH compared to normoxic controls. Cyclophilin was not different between groups.

CONCLUSIONS: The LTH fetal adrenal cortex has increased capacity for cholesterol ester uptake via LDLR as well as increased capacity for de novo synthesis of cholesterol from acetate (HMG-CoAR). Considering the low capacity for cholesterol uptake and synthesis in the fetal adrenal cortex compared to the adult, the increased expression of these key genes may provide for the enhanced acute capacity for cortisol synthesis despite lower steroidogenic enzyme expression. (NIH grants HD31226, P20-MD001632)

S-061

Distinct Molecular Pathways in Endometriosis-Associated Ovarian Cancer. Yanett Anaya,[1] Shikha Khatri,[1] Weimin Xiao,[2] Matthew L Anderson,[1] Shannon M Hawkins.[1] *[1]Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Chemistry, University of Houston, Houston, TX, USA.*

Background: Studies have shown ovarian cancer arising from endometriotic lesions, specifically clear cell and endometrioid ovarian cancers. The molecular mechanisms by which carcinomas arise in endometriosis and how they differ from non-endometriosis associated ovarian cancers, is not presently known. **Objective:** To determine key molecular pathways that differ in endometriosis-associated endometrioid ovarian cancers (EAEOC) compared to endometrioid cancers (EOC). **Methods:** After IRB approval, RNA was extracted from frozen tissue of EAEOC (n=4) and EOC (n=4) and sent for whole genome expression analysis, using Illumina's Human WG-6 version 3.0 BeadChips. Pathway analysis of differentially expressed genes was performed using the Ingenuity Pathway Analysis tool. For gene expression validation studies, independent samples of RNA were reverse transcribed and real-time quantitative PCR was performed using TaqMan assays. The relative quantity of transcript was calculated using the $2^{-\Delta\Delta C}{}_{T}$ method and plotted as mean± SEM. Student's *t* test was used to generate *P* values. Hierarchical clustering was performed on gene expression profiles from 5 endometriomas, 4 proliferative phase endometrium, 4 EOC, and 6 EAEOC. **Results:** Whole genome expression analysis revealed 758 unique probes were differentially expressed (>2.0 fold change, $p<0.05$). The top 4 canonical pathways represented by these differentially expressed genes were Amyloid Processing, BMP Signaling, PPARα Activation, and Molecular Mechanisms of Cancer. Taqman assay confirmed dysregulated expression of specific gene products in the BMP signaling pathway with decreased levels of BMP8B ($p=0.008$), MAPK13 ($p=0.03$), MAPK14 ($p=0.004$), and PRKAG2 ($p=0.02$) in EAEOCs compared to EOCs. Our hierarchical analyses indicate that specimens of EAEOC were distinguished from EOC and appear most similar to specimens of endometriomas and endometrium. **Discussion:** Our data indicate that patterns of gene expression in EAEOC are distinct from those found in EOC and are consistent with an origin for EAEOC in endometriotic implants. Also, our results identify signaling pathways that appear to uniquely contribute to the pathogenesis of this subset of ovarian cancers. Future studies will focus on dissecting the role of these pathways and determining how best to utilize this insight to improve outcomes for women with EAEOC.

S-062

Expression of the *N*-Methyl-*D*-Aspartate Receptor, a Precursor to Anti *N*-Methyl-*D*-Aspartate Receptor Encephalitis, Is Not Limited to the Neuronal Tissue of Ovarian Teratomas. Rachel M Clark,[1] Lawrence Zukerberg,[3] Maureen Lynch,[1] Bo Rueda.[1] *[1]Vincent Center for Reproductive Biology, Massachusetts General, Boston, MA, USA; [2]Pathology, Massachusetts General, Boston, MA, USA.*

Introduction: Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is one of the most common limbic encephalitides. Approximately 80% of patients with anti-NMDAR encephalitis are women; 50% are associated with an ovarian teratoma. Previous studies suggest expression of NMDAR on teratoma neural tissue initiates an autoimmune response to NMDAR in the brain. As the teratomas of some patients with anti-NMDAR encephalitis contain little or no neural tissue, we questioned if there could be another source of NMDAR expression. Our objective was to assess the presence of NMDAR and correlate its expression with histology of teratomas resected for usual surgical indications as well as teratomas resected from patients with anti-NMDAR encephalitis.

Design: We identified all teratomas resected after January 1, 1990 and obtained paraffin embedded tumors from 13 patients (3 cases with associated encephalitis and 10 cases without) for immunohistochemical analysis with rabbit anti-NMDAR subunit NR1, rabbit anti-NMDAR subunit NR2B and mouse anti-MAP2. Immunoreactivity was graded by an institutional pathologist and scored in intensity. Histology and relative proportions of each type of tissue present in all teratomas was recorded.

Results: Of the 10 control teratomas, 50% contained neural tissue expressing one or both NMDAR subunits. Of the 3 case teratomas, two contained scant neural tissue which expressed both NMDAR subunits. Despite extensive sampling there was no neural tissue found in the third case. All 13 teratomas were characterized by large amounts of squamous tissue expressing one or both NMDAR subunits, with the three cases displaying intense positivity. In one of the cases, this was the only teratomatous tissue that was positive for NMDAR.

Conclusions: There was no difference in expression of NMDAR bearing neural tissue in the teratomas of cases or controls. All three cases had intense expression of NMDAR by squamous tissues, and in one case this was the only NMDAR bearing tissue. Our data suggest that if NMDAR bearing

neural tissue is a causal factor of anti-NMDAR encephalitis, it is not the only one. NMDAR expression by squamous tissue may also contribute to the development of anti-NMDAR encephalitis and may do so in the absence of teratomatous neural tissue.

S-063

Calcitriol Promotes TLR9/NOD2 Mediated Clonal Expansion, Survival and Th1 Differentiation of Antigen Specific CD8$^+$ T-Cells for Ovarian Tumor Control. Sayeema Daudi, Cheryl Eppolito, Junko Matsuzaki, Shashikant Lele, Kunle Odunsi. *Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA.*

Introduction

A major limitation of current immunotherapy approaches in ovarian cancer is the inability to generate robust pools of effector and memory T-cell responses for controlling tumor growth. Calcitriol acts as a potent immune system regulator via intracellular calcium influx and subsequent activation of CD8$^+$ T-cells. MIS416 is a novel adjuvant that signals via the TLR9/NOD2 pathway, and comprises of muramyl dipeptide repeats and native bacterial DNA fragments. We hypothesize that calcitriol would endow MIS416 induced antigen specific CD8$^+$ T-cells for enhanced clonal expansion, Th1 effector function and memory differentiation.

Methods

OVA-specific CD8$^+$ T-cells were obtained from the lymph nodes (LN) of OT1 TCR transgenic Rag2$^{-/-}$ mice (OT1-cells). OT1-cells were stimulated with plate-bound anti-CD3/B7.1 in the presence or absence of calcitriol and/or MIS416. Activation, differentiation and apoptotic cell death were estimated by flow cytometry in the cultured cells. Next, the *in vivo* efficacy of calcitriol in the presence or absence of MIS416 was examined by adoptive transfer of CFSE-labeled OT1-cells i.v. into syngeneic recipients (n=5 per group). Immunization with OVA protein admixed with incomplete freund's adjuvant (IFA), calcitriol, MIS416 or the combination of calcitriol and MIS416 were administered to the mice on day 0 and 7. OT1-cells from LN and spleen were analyzed following stimulation with OVA peptide.

Results

In vitro, calcitriol and MIS416 treatment enhanced OT1-cell effector function compared to controls. Increasing doses of calcitriol rescued OT1-cells from cell death, and IFN-γ production from OT1-cells was augmented by calcitriol in MIS416 treated cells in a dose dependent manner. *In vivo*, MIS416 alone or in combination with calcitriol induced a 6 fold increase in IFN-γ production compared to IFA. Moreover, OT1-cell expansion in the MIS416 plus calcitriol treated mice persisted until day 14, indicating superiority of this combination in generating durable immunity.

Conclusions

Calcitriol augmented MIS416 mediated CD8$^+$ T-cell activation, proliferation, effector differentiation and survival, thereby leading to long lasting immunity. These studies lay the foundation for a future clinical trial of the combinatorial approach of calcitriol/MIS416 for immunization in ovarian cancer patients.

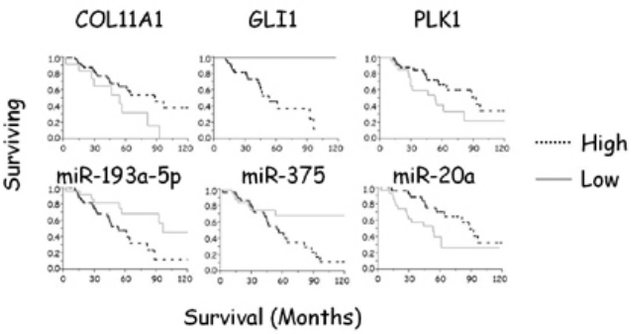
S-064

Nanofluidic Technologies Identify Clinical Features of Neoadjuvant Treatment and Outcome in Ovarian Cancer. Cristiano Ferlini, Marisa Mariani, Amanda Wilmot, Shohreh Shahabi. *Reproductive Tumor Biology Lab, Danbury Hospital, Danbury, CT, USA.*

Objectives: Nanofluidic technologies allow an incredible increase in the throughput and quality of translational studies, by allowing the direct comparison of the expression of microRNAs, genes and SNPs. This work was aimed at validating the use of this technology in ovarian cancer patients.

Methods: A set of 78 ovarian cancer patients was analyzed. Follow up data was available for all the patients and 15/78 underwent neoadjuvant chemotherapy. A chip 48.48 served to analyze a panel of 96 potential predictors of the outcome and response to chemotherapy. Overall survival was analyzed using Kaplan-Meier method and comparisons between groups by log-rank test. Statistical significance was determined at a level of $p < 0.05$.

Results: We assessed the impact of neoadjuvant chemotherapy on the selected genes and microRNAs. A MANOVA was performed and the factors sensitive to neoadjuvant treatment were identified with specific post-hoc analysis. Among the genes MKI67, PLK1, PBK were dramatically reduced. This finding demonstrates that chemotherapy has a huge impact mainly in proliferating cells. We noticed deactivation of the TUBB3 survival pathway, which was reduced while its negative regulator GNAI1 was increased. ERBB2 resulted downregulated in the neoadjuvant setting, while HGF and PTEN were increased. We noticed a huge effect on microRNAs, whose expression broadly increased, except miR-20a and miR-141. At the gene level the most potent predictor for outcome were COL11A1, GLI1 and PLK1.



Patients exhibiting high levels of COL11A1 and PLK1 were poor performers, while the opposite occurred for GLI1. At the micro-RNA level the best predictors were miR-193a-5p, miR-375and miR-20a. For the first two, the poor performers were featured by high levels while the opposite was evident for miR-20a.

Conclusions: Our results in the neoadjuvant setting suggest that patient after surgery can benefit by treatment with biological agents targeting HGF but not ERBB2. Moreover, this technology will open new avenues to upfront stratify patients most likely refractory to standard treatment.

S-065

Dicholoroacetate Increases Sensitivity to Chemotherapy Treatment of Epithelial Ovarian Cancer Cells. Nicole M Fletcher, Jimmy Belotte, Michael P Diamond, Ghassan M Saed. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*

Objective: Ovarian cancer is the leading cause of death from gynecologic malignancies. Despite the fact that many patients with advanced disease will respond to initial chemotherapy, the majority will develop resistance. Evidence supporting the role of oxidative stress in the prognosis and metastasis of cancer is overwhelming. Oxidative stress triggers cancer cells to favor glycolysis, and gives rise to enhanced lactate production. Dichloroacetate (DCA) is known to convert anaerobic to aerobic metabolism. The objective of this study was to determine whether DCA treatment of epithelial ovarian cancer (EOC) cells would increase the sensitivity to chemotherapeutic agents.

Methods: Cell viability was assessed with the trypan blue exclusion method using TOV112D EOC cells and its cisplatin resistant (0.8 ug/ml) counterpart following treatment with DCA (5 ug/day), over the course of seven days via an osmotic pump. Apoptosis was assed with as a function of caspase-3 levels using real-time RT-PCR in three additional EOC cell lines (MDAH-2774, Ov21, and OV433) and their chemoresistant counterparts (docetaxel or cisplatin). Data were analyzed using a mixed-design analysis of variance (ANOVA) model with treatment condition and cell line as between factors, and exposure day as within factor.

Results: As compared to controls, there was a decrease in viability after day three through six in TOV112D EOC cells (to 94.1, 87.9, 86.3, and 86.2% viable, $p<0.05$) and in day four through seven in its cisplatin resistant counterpart (to 82.1, 77.7, 61.2, and 58.5%, $p<0.05$). As compared to their chemosensitive counterparts, there was a decrease in caspases-3 mRNA levels from 0.122 to 0.0279, from 0.116 to 0.0155, and from 0.149 to 0.0264 ng/ug RNA in chemoresistant EOC cells Ov21, MDAH-2774, and OV433, respectively ($p<0.05$).

Conclusion: Shifting anaerobic to aerobic metabolism using DCA increases the sensitivity of EOC cells to chemotherapeutic agents. DCA or other similar agents, may thus serve as future therapeutic agents to decrease chemoresistance, thereby providing the opportunity for improved outcomes.

S-066

The Effect of Microtubule Stabilizing Agents on Ovarian Cancer Cells Harboring Hot Spot Mutations of *p53* . Lisa Fusco,[1] Chia-Ping H Yang,[2] Gerda Hofstteter,[2] Robert N Samuelson,[1] Shohreh Shahabi.[1] *[1]Department of Obstetrics, Gynecology and Reproductive Biology, Danbury Hospital, Danbury, CT, USA; [2]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA.*

Background: Epothilone and Taxol are able to induce the accumulation and phosphorylation of p53, a tumor suppressor protein that has been shown to play a role in apoptosis. Post-translational modifications of p53, including phosphorylation and acetylation stabilize and activate the p53 protein. The

signaling pathways that impinge on p53 after treatment are still not well defined. Missense mutations in *p53* frequently occur at 'hotspot' amino acids which are highly conserved and represent regions of structural or functional importance. In human ovarian carcinomas, the *p53* tumor-suppressor gene is frequently mutated. The impact of hot spot mutations of *p53 in response to microtubule stabilizing agents were evaluated.*

Methods: In order to evaluate the effect of different hot spot mutations of p53 in response to epothilone B, Ixabepilone and Taxol, the human ovarian wild-type (wt)-p53 cell line A2780 which were stably transfected with an empty vector (CMV), with m-175-, m-248- or m-273-p53 were used. IC_{50} of these p53 mutant cell lines for the above microtubule stabilizing agents were determined. The expression p53, phosphorylated p53 (Ser15), acetylated p53, and the p53 protein family member TAp73 and ΔNp73 were investigated by western blot analyses. The expression of p53 target genes, such as p21, GADD45, BAX, PIDD, NF-kB2 and PAI-1, were evaluated by RT-PCR.

Results: The p53 mutant m-248 is 2.3-fold resistant to epothilone B and m273 is 3-fold resistant to Taxol. M-248 was not acetylated after treatment with epothilone B, compared to all other cell lines. P21 and PAI-1 were induced by epothilone B in A2780 cells. Expression of p21, GADD45 and PAI-1 in mutant cell lines was generally reduced.

Conclusions: These data provide evidence for the first time that ovarian cancer cells harboring mutant p53 were resistant to microtubule stabilizing agents, such as Taxol and epothilone B. It is suggested that the hot spot mutation of p53 may be involved in the pathways leading to Taxol resistance in ovarian cancer.

S-067

Developmentally Restricted Differentiation Antigens Are a Target for Immunotherapy in Epithelial Ovarian Carcinoma. Heidi Godoy,[1] Amy Beck,[1] Paulette Mhawech-Fauceglia,[2] Anthony Miliotto,[1] Austin Miller,[3] Shashi Lele,[1] Kunle Odunsi.[1] *[1]Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA; [2]Pathology, University of Southern California, Los Angeles, CA, USA; [3]Statistics, University at Buffalo, Buffalo, NY, USA.*

Background

Developmentally restricted differentiation antigens or cancer placental antigens, tastin and bystin, are expressed in normal human placental tissue. Tastin and bystin are components of an adhesion molecule that plays a critical role in the implantation of the embryo to the uterus. Cell adhesion molecules have been implicated in the metastasis of carcinomas and could be critical targets for immunotherapy in epithelial ovarian carcinomas (EOC). Our objective was to define the frequency of expression of tastin and bystin and correlate their expression with outcome.

Methods

Expression of tastin and bystin mRNA in a panel of human tissues and 70 EOC specimens was investigated using qualitative polymerase chair reaction (PCR). Amplification products were confirmed by sequencing. Validation of results was performed using IHC analysis of tastin and bystin applied on a tissue microarray (TMA) of 202 EOC tissues. The distribution of tastin and bystin expression and clinico-pathologic variables were analyzed. Survival probabilities were estimated by Kaplan-Meier method and statistical significance was determined by log-rank test.

Results

Expression of tastin and bystin was restricted to placental and testis tissue. Of the 70 EOC specimens tested with PCR, 62/70 (89%) and 66/70 (94%) expressed tastin and bystin, respectively. Tastin and bystin expressions were observed in 139/202 (68.8 %) and 162/202 (80.2 %) of specimens on the TMA, respectively. Tastin and bystin expression in stage I/II disease was 66% and 67% compared to 69% and 81% in stage III/IV disease, respectively. Grade 3 tumors tended to have higher expression of both tastin and bystin; however, the difference was not statistically significant. Bystin expressing tumors were more likely to have tastin expression (p 0.001), though there were no other correlations observed between the two antigens. The presence of bystin and tastin did not have a statistically significant effect on overall survival.

Conclusion

The tissue-restricted expression of tastin and bystin, their abundant expression in EOC and advanced stage disease make these developmentally restricted antigens an attractive target for antigen-specific immunotherapy in EOC.

S-068

Characterization of AKAP13 and Chemotherapy Resistance in Ovarian Cancer Cells. Ashley N Lawler, Paul H Driggers, James H Segars. *Program in Adult and Reproductive Endocrinology, NICHD, NIH, Bethesda, MD, USA.*

Background: AKAP13, a Rho-guanine nucleotide exchange factor (Rho-GEF) overexpressed in prostate cancer, familial breast cancer, and ovarian cancer, has been identified as a predictor of resistance to tipifarnib, a competitive farnesyltransferase inhibitor used in the treatment of acute myelogenous leukemia. Although studies have associated AKAP13 overexpression with drug resistance in leukemia, the effect of AKAP13 expression in ovarian cancer cells remains unknown.

Objective: The objective of this study was to assess the effect of AKAP13 on resistance to chemotherapeutic agents cisplatin, a platinum-containing alkylating agent, and paclitaxel, a microtubule-stabilizing agent, in CAOV-3, OVCAR-3 and SKOV-3 ovarian cancer cell lines.

Methods: OVCAR-3 cells were transfected with increasing amounts of AKAP13 expression vector and cell proliferation was assayed by addition of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] (Promega), and incubation at 37°C for two hours. This experiment was repeated with cisplatin treatment following AKAP13 transfection. CAOV-3, OVCAR-3, and SKOV-3 cells were treated with siRNA to reduce AKAP13 expression, and were then treated with increasing doses of cisplatin or paclitaxel and cell survival was assayed. Positive controls established specific reduction of AKAP13 protein levels with siRNA, versus All-Stars Negative negative control siRNA (Qiagen).

Results: Overexpression of AKAP13 by transfection in OVCAR-3 cells dose-dependently increased cell survival, and rescued viability of cells treated with cisplatin, consistent with the observation that AKAP13 increases resistance to chemotherapy. Knockdown of AKAP13 in CAOV-3 cells by treatment with siRNA decreased cell survival after treatment with paclitaxel and thus increased the effect of the chemotherapy. Similarly, knockdown of AKAP13 in CAOV-3 cells by siRNA treatment decreased cell survival after treatment with paclitaxel, increasing the apoptotic effect of the drug.

Conclusions: The level of AKAP13 expression level in two ovarian cancer cell lines was correlated with response to chemotherapeutic agents, cisplatin and paclitaxel. Thus, overexpression of AKAP13 in ovarian tumors might contribute to chemotherapy resistance. Future studies will examine the correlation between AKAP13 expression level and tumor response to cisplatin or paclitaxel.

S-069

Development of Fluorescence Correlation Spectroscopy (FCS) as a Screening Tool for Ovarian Cancer. Jacob Rotmensch, Liaohai Chen. *Gynecologic Oncology, Rush University Medical Center, Chicago, IL, USA.*

FCS is a powerful tool that has the potential to characterize a serum marker at the molecular level based on its physical properties. It can qualitatively and quantitatively examine a single molecule that is labeled with fluorescence at nanomolar concentrations. This tool may be useful in improving the ability to detect and differentiate serum markers from benign and malignant tumors. In this study, we analyzed the tumor marker CA-125 with FCS. CA-125 from 50 patients, 25 with benign and 25 with malignant epithelial tumors,were blindly analyzed. The CA-125 in malignant samples ranged from 77 to 270 units/ml and in benign samples from 27 to 130 units/ml.

Analysis of samples after fluorescent labeling by FCS showed that the OD@450nm was lower (0.072-0.196) for all benign ovarian tumors compared to malignant epithelial ovarian (0.45-0.81). Autocorrelation curves from FCS to determine the diffusion times of the molecules showed longer times for the carcinomas than for the benign tumors indicating that the CA-125 from malignant tumors are larger than that from benign tumors.

In conclusion, the use of FCS may be a very sensitive method to analyze serum markers at the nanoscale. It has the potential to determine physical properties of single molecules and to differentiate markers obtain from benign and malignant disease based on physical properties. It is a very powerful tool in detecting tumor markers at the molecular level.

S-070

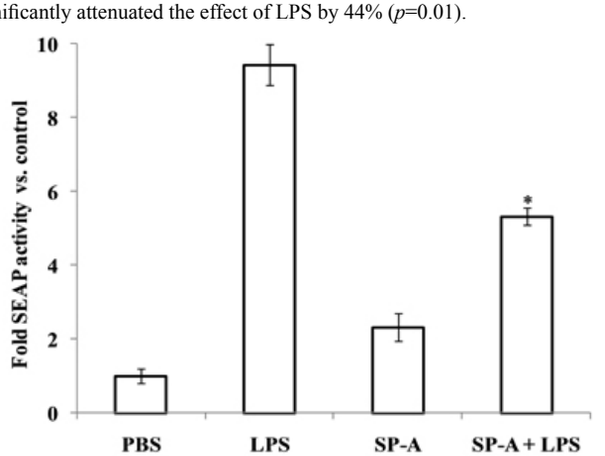
Evaluation of Response to Microtubule-Interacting Agents Using Primary Epithelial Ovarian Cancer Cells from Ascitic Fluid. Charis Venditti, Ilenia Pellicciotta, Robert N Samuelson, Shohreh Shahabi. *Department of Obstetrics, Gynecology and Reproductive Biology, Danbury Hospital, Danbury, CT, USA.*

Background: We developed five primary epithelial ovarian cancer cell lines (SS2 to SS6) from ascites in patients with histologically confirmed epithelial ovarian cancer. The ex-vivo chemosensitivity was tested in these cell lines

with microtubule stabilizing agents (MSA): Taxol, epothilone B (EpoB), and discodermolide. Mutation in tubulin is one of the mechanisms of resistance to MSA and p53 status correlates with MSA sensitivity. We performed α-β tubulin and p53 mutation analyses to test this hypothesis.
Methods: Primary ovarian cancer cells were isolated from ascitic fluid. Anti-Cytokeratin cocktail AE1+AE3 antibodies were used to confirm the purity of epithelial cancer cells. IC_{50} of ovarian cancer cells with MSA was determined. Cell cycle analyses of the primary ovarian cancer cells treated with MSA were determined with 4-1000nM for 24 h.
Results: α-β tubulin and p53 were amplified by RT-PCR and sequenced. Anti-Cytokeratin confirmed the purity of epithelial ovarian cancer cells. Primary cancer cells were not completely blocked at the G2/M phase by 200nM MSA in 24 h, although they were highly sensitive to Taxol, EpoB, and discodermolide. Doubling time was 72 hours for the primary cancer cells. α-β tubulin mutation was not found in the primary ovarian cancer cells. SS3 and SS6 harbor p53 mutations at residue 72 (Arg to Pro). Arg 72 is located in the transcriptional transactivation domain.
Conclusion: The primary epithelial ovarian cancer cells have a longer doubling time compared to immortalized ovarian cancer cell lines. The clinical significance of p53 codon 72 polymorphism in epithelial ovarian cancer requires further study.

S-071

Surfactant Protein (SP)-A Attenuates LPS-Induced NF-κB Activation in a Mouse Macrophage Reporter Cell Line. Varkha Agrawal,[1] Keith Smart,[1] Yana Filipovich,[1] Emmet Hirsch.[1,2] *[1]Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, IL, USA; [2]Ob/Gyn, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA.*
Objective: Surfactant protein (SP)-A is produced in fetal lung and may either activate or suppress inflammatory responses. We have demonstrated that SP-A suppresses the LPS-induced production of proinflammatory cytokines in macrophages via a toll-like receptor-2 mediated mechanism. Here we examine the effect of SP-A on LPS-induced nuclear factor (NF)-κB activation in a mouse macrophage reporter cell line.
Methods: RAW-Blue™ (InvivoGen, San Diego, CA) is a mouse macrophage cell line that stably expresses a secreted embryonic alkaline phosphatase (SEAP) gene inducible by the NF-κB transcription factor. RAW-Blue cells (4×10^5/well) were cultured in 12-well plates and stimulated with lipopolysaccharide (LPS, 5ng/ml) for 4 hours in the presence or absence of SP-A (20μg/ml). Supernatants were incubated with a detection substrate (QUANTI-Blue™, InvivoGen) at 37°C for 30 min, and relative SEAP levels were determined using a spectrophotometer at 620-655 nm.
Results: LPS increased SEAP activity above baseline at 4 hours. SP-A significantly attenuated the effect of LPS by 44% (p=0.01).



Discussion: SP-A significantly suppresses LPS-induced NF-κB activation in a mouse macrophage reporter system, paralleling observations previously made for various downstream inflammatory markers in mouse peritoneal macrophages. The RAW-Blue system may serve as a convenient, rapid screen for assessing the effects of various pregnancy-related compounds on the activation of NF-κB without having to resort to protein assays or RNA extraction and PCR.

S-072

Probiotic Attenuation of Inflammatory Responses in Amnion: A Possible Role in the Prevention of Preterm Labor? R Koscik,[1] G Reid,[2] W Li,[1] A Martins,[2] S Kim,[2] AD Bocking,[1] JGR Challis.[1] *[1]Department of Obstetrics and Gynecology, University of Toronto; [2]Lawson Research Institute, University of Western Ontario.*
We have reported previously that addition of supernatant from the probiotic Lactobacillus rhamnosus GR-1 to human amnion cells in culture, attenuates their stimulated output of TNF_α, in response to lipopolysaccharide (LPS). We now test the **hypothesis** that GR-1 supernatant not only abrogates the pro-inflammatory response, but also enhances the output of anti-inflammatory mediators by these cells. **Methods:** We maintained amnion cells obtained from women at term caesarean section, in the absence of infection, in a mixed cell monolayer culture in the presence of LPS, lipoteichoic acid (LTA), GR-1 or combinations of these. We determined outputs of cytokines and chemokines after various times in culture by using the Bio-Plex-27 assay and ELISA. Results: Measurements of lactate dehydrogenase showed that none of the treatments had any significant cytotoxic effects. As reported previously, LTA and LPS stimulated the output of TNF_α. This response was blocked with GR-1 although GR-1 had no effect on its own. We did not detect significant output of $IL\text{-}1_\beta$ with any treatment. Basal outputs of IL-10 and IL-4 were below detection limits and neither of these anti-inflammatory cytokines was increased above basal with any treatment. GR-1, but not LPS or LTA significantly increased the output of monocyte chemo-attractant protein, MCP-1. There was a similar effect on output of RANTES, stimulation with GR-1 +/- LPS or LTA, but no effect of LPS or LTA alone. The output of IFN_γ, was increased by GR-1 alone or in the presence of LPS or LTA; but there was no effect on IFN_γ of LPS or LTA alone; Granulocyte-macrophage colony stimulating factor, GM-CSF was increased by LTA or LPS + GR-1, but not GR-1 alone. We **conclude** that probiotic supernatant activity can alter the cytokine response to endotoxin stimulation by mixed cultures of amnion cells. The ratio of pro- to anti-inflammatory cytokine output is altered by reducing output of TNF_α, without the increase in IL-10 that we found in trophoblast cells. GR-1 is also conducive to recruitment of monocytes and neutrophils through stimulation of MCP-1, GM-CSF, RANTES and IFN_γ. Our results support a role for GR-1 in modulating inflammatory responses of amnion, and might suggest an opportunity for the use of probiotic organisms in ameliorating inflammation associated preterm labor.

S-073

Intraperitoneal Surfactant Protein A (SP-A) Suppresses PGN/Poly (I:C)-Induced Preterm Delivery in Mice. Yana Filipovich,[1] Varkha Agrawal,[1] Emmet Hirsch.[1,2] *[1]Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, IL, USA; [2]Obstetrics and Gynecology, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA.*
Objective: Surfactant protein A (SP-A), an endogenous protein found in amniotic fluid and produced by fetal lung, is capable of either activating or suppressing inflammatory responses. Toll-like receptors (TLRs) are membrane-bound receptors that recognize molecular constituents of pathogens and initiate the innate immune response. Here we test the impact of SP-A on preterm delivery induced by simultaneous intraperitoneal (IP) administration of peptidoglycan (PGN, a TLR2 ligand) and polyinosinic:cytidylic acid (poly(I:C), a TLR3 ligand).
Methods: CD-1 mice (12 per group) on day 14.5 of gestation underwent IP injection of both PGN (0.9mg/mouse) and poly(I:C) (3mg/mouse) with or without supplemental SP-A (225 or 450 μg/mouse). A separate group of animals (n=5) underwent IP injection with SP-A alone (225 μg/mouse). Mice were observed after surgery for preterm delivery (delivery of at least one pup within 48 hours).
Results: IP injection of SP-A alone did not cause preterm delivery in any mice. IP injection of both PGN and poly(I:C) caused preterm delivery in 92% of mice. The addition of 225 μg or 450 μg of SP-A to PGN/poly(I:C) significantly decreased preterm delivery to 67% and 42%, respectively (P=0.002).
Conclusions: In the mouse model, SP-A suppresses preterm delivery induced by intraperitoneal administration of PGN/poly(I:C) in a dose-responsive fashion. This result resembles the effect of SP-A after intrauterine administration of either lipopolysaccharide (LPS) or PGN/poly(I:C) (SGI 2011, abstract #O-063), but is opposite to its effect with intraperitoneal LPS (SGI 2011, abstract #F-232). The effect of SP-A thus appears compartment- and TLR ligand-specific.

Summary of compartment- and ligand-specific effects of SP-A on preterm delivery rate*

	Intraperitoneal injection		Intrauterine injection	
	PGN/poly I:C (present study)	LPS (SGI2011 F-232)	PGN/poly I:C (SGI2011 O-063)	LPS (SGI2011 O-063)
No SP-A	92%	25%	73%	90%
Plus SP-A	42%	75%	18%	38%
p-value	0.03	0.04	0.02	0.03
SP-a effect on preterm delivery	↓	↑	↓	↓

*Doses of reagents vary based on the specific experiment.

S-074

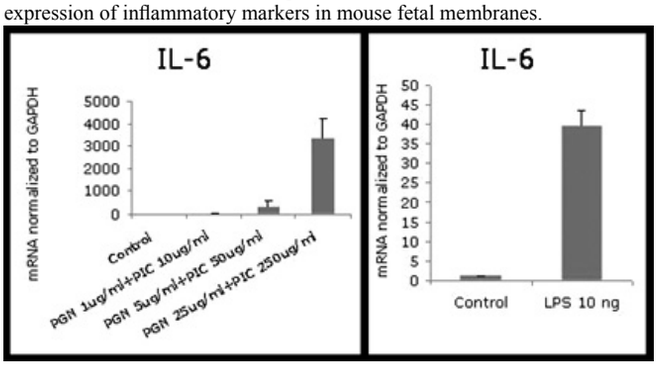
A Method for Explant Culture of Mouse Fetal Membranes. Yana Filipovich,[1] Emmet Hirsch.[1,2] *[1]Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, IL, USA; [2]Obstetrics and Gynecology, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA.*

Objective: The purpose of this study was to optimize a mouse fetal membrane explant culture system.

Methods: Fetal membranes were collected from pregnant mice on day 14.5 of gestation and were briefly washed in PBS. Circular pieces of membranes ('disks') were cut from the whole using a skin biopsy punch (3mm, 4mm or 6mm). Two to three membrane disks per well were placed in a Falcon 24-well tissue culture plate containing Dulbecco's modified Eagle's medium with Ham's F12 nutrient mixture (1:1) with 50 units/ml penicillin, 50µg/ml streptomycin and 15% heat inactivated fetal bovine serum. Disks were cultured for up to 48 hours at 37°C in 5% CO_2 in humidified air to allow for recovery. Following this, medium was replaced with either fresh medium, medium containing lipopolysaccharide (LPS, 10ng/ml to 15µg/ml) or medium containing a mixture of peptidoglycan (PGN, 1µg/ml to 25µg/ml) and polyinosinic:cytidylic acid (poly I:C , 10µg/ml to 250µg/ml). This was followed by further incubation for up to 8 hours prior to extraction of total RNA. Real time-PCR was performed for IL-6, IL-1β and TNF-α, normalized to the housekeeping gene glyceraldehyde 3 phosphate dehydrogenase (GAPDH).

Results: Fetal membrane explants retained normal morphology over a 48-hr incubation period. Continued production of mRNA was demonstrated by GAPDH PCR, providing evidence of tissue viability. Baseline cytokine levels were stable between 24 and 48 hours after plating, and therefore exposure to test substances was performed at 24 hours. LPS induced the expression of IL-6, IL1-β and TNFα mRNA at 4hr but not 1hr with exposures as low as 10ng/ml. No dose-response effect was seen above 10ng/ml LPS. PGN plus poly(I:C) induced IL-6 and TNFα mRNA at 4 hours, with dose-responsive expression of IL-6 ($p<0.001$).

Conclusion: We demonstrate an organ culture method to measure the stimulated expression of inflammatory markers in mouse fetal membranes.



S-075

Amniotic Fluid (AF) Analysis To Confirm Suspected Intrauterine Infection/ Inflammation (IUI): Is It Helpful? Catherine E Ford, Mehmet R Genc. *Obstetrics and Gynecology, Brigham & Women's Hospital, Boston, MA.*

Background: Clinical signs and symptoms of IUI (fever, maternal and/or fetal tachycardia, purulent or foul-smelling AF or vaginal discharge, uterine tenderness, and maternal leukocytosis) are nonspecific and subjective. For this reason, some clinicians prefer to obtain AF by amniocentesis and analyze it to confirm the diagnosis of IUI. Our objective was to compare a strategy utilizing AF analysis vs one based on clinical signs and symptoms alone for the management of patients suspected of IUI.

Method and Materials: We compared maternal and neonatal outcomes of 40 singletons who underwent amniocentesis and randomly chosen 40 singletons whose management did not involve amniocentesis for suspected IUI. Patients who presented after 16 weeks with at least 1 of the following symptoms, uterine tenderness, maternal fever, maternal tachycardia and/or fetal tachycardia, were included in the study. The effect of AF analysis on outcomes was controlled for characteristics identified to be different by the univariate analysis.

Results: There were more cases of preterm labor with intact membranes (3% vs. 23%) and PPROM (35 vs. 85%) in the no-amniocentesis group ($p<.01$). There was no difference between the maternal and neonatal outcomes except the duration of pregnancy from admission to delivery, which was longer in the amniocentesis group (Table). However, amniocentesis was no longer associated with prolonged pregnancy after controlling for preterm labor and PPROM.

Conclusions: Amniocentesis and AF analysis does not offer a significant advantage over management based on clinical signs and symptoms alone in patients suspected of IUI. This highlights the need for diagnostic modalities to identify IUI in asymptomatic patients for timely intervention.

	Amniocentesis N=40	No amniocentesis N=40	P
Composite outcome[a]	16	20	NS
Fetal death>20wk	6	8	NS
Fetal death<20wk	1	1	NS
Maternal death	0	0	...
Maternal Sepsis	1	3	NS
Bronchopulmonary dysplasia (BPD)	8	10	NS
Necrotizing enterocolitis(NEC)	1	3	NS
Periventricular leukomalacia (PVL)	0	1	NS
Intraventricular hemorrhage (IVH), grade 3 and 4	0	0	...
Postpartum endometritis	3	1	NS
Gestational age at delivery [b]	32(19-41)	29(17-40)	NS
Days from admission to delivery [b]	18(0-135)	5(0-139)	<.01

[a] maternal sepsis, maternal death, perinatal death, neonatal sepsis, BPD, NEC, PVL and IVH grades 3 and 4; [b] median (range); NS: not significant

S-076

Chlamydia and Gonorrhea Infection Does Not Increase the Risk of Preterm Delivery. Meg Hill,[1] Paul C Browne,[1] Menon Seema,[1] Sarah Smith,[1] Zhang Hongmei,[2] Tong Xin.[2] *[1]Obstetrics and Gynecology, Palmetto Health Richland, Columbia, SC, USA; [2]Statistical Analysis, University of South Carolina, SC.*

Objective:

The objective of this study was to determine whether prenatal infection with Gonorrhea and/or Chlamydia increases the risk of preterm delivery in an indigent metropolitan clinic.

Study Design:

This was a retrospective cohort study. We requested all charts for patients presenting for prenatal care at the USC/Palmetto Health Womens' Center between 06/01/2006 and 08/31/2009. The chart analysis covered 06/01/2006 to 12/31/2007. We analyzed the data for 1722 pregnancies, 600 charts were excluded for lack of outcome data. 1122 patients (65.2%) met study criteria. Essential study data included at least on Chlamydia/Gonorrhea (GCC) PCR screen and outcome data for the pregnancy. Chi Square analysis was used for binomial data.

Results:

There were 935 patients (83.3%) with negative GCC PCR and 187 (16.7%) with at least one positive PCR. Of the 187 with a positive PCR, 49 (26.2%) had multiple positive PCR screens.

Positive GCC PCR was more common in African American patients (22.8%) than in whites (5.7%) or Hispanics (7.6%). Positive GCC PCR was more common in primigravid patients (21.0%)than in multigravid patients (14.7%). Positive GCC PCR was more common in non-married patients (18.8%) than in married patients (4.3%).

The overall percentage of term delivery did not differ between the GCC positive and negative groups (83.4% and 82.2% respectively, P=0.6905). The rate of preterm premature rupture of membranes (PPROM) was not statistically different between groups (positive 2.67%, negative 3.97%, P value 0.3960). Preterm labor with preterm delivery was also not statistically different between groups (positive 8.02%, negative 8.25%, P=0.9162).

There was a trend toward a higher risk of cesarean delivery in the group of patients with negative GCC PCR, though this did not meet statistical significance (positive 17.11%, negative 23.26%, P=0.0651).

Conclusions:

Maternal infection with Chlamydia and/or Gonorrhea is not associated with an increased risk of PPROM or PTL. There is a trend toward a higher rate of cesarean delivery in patients with a negative Chlamydia/Gonorrhea PCR.

Pregnancy outcome by GCC PCR status

	GCC Positive	GCC Negative	P Value
Term Delivery	83.42	82.21	0.6905
PTL	8.02	8.25	0.9162
PPROM	2.67	3.97	0.3960
Cesarean Delivery	17.11	23.26	0.0651

S-077

PDE Inhibitors: A Treatment for Mothers and Babies at Risk of Preterm Delivery. Laura Howe,[1] Johann Malawana,[1] Renyi Hua,[1] Bronwen Herbert,[1] Simon Waddington,[2] Mark Johnson.[1] *[1]Surgery & Cancer, Hammersmith Hospital Campus, Imperial College London, London, United Kingdom; [2]Institute for Women's Health, UCL, London, United Kingdom.*

Premature delivery is the most important problem in obstetrics causing over 70% of neonatal death and handicap. Although some treatments reduce the risk of preterm labour (PTL), none have been shown to improve neonatal outcomes. Cyclic adenosine monophosphate (cAMP) has been shown to down-regulate the oxytocin receptor and is also known to be an immunomodulator, inhibiting both NFkB-driven transcription and mitogen activated kinase (MAPK) activation, suggesting that cAMP agonists may be ideal agents for the prevention of PTL. Some data support the role of phosphodiesterase 4 (PDE) inhibitors in the management of PTL, but the role of PDE3 has not been assessed. PDE3 is abundant in embryonic neuroepithelium, and PDE3 inhibitors have been found to have neuroprotective effects in cultured neurons. We administered 10μg of LPS into the right horn of the uterus, at laparotomy, performed on E16 of gestation in CD1 outbred mice. 2 hours prior to this we administered 2mg/kg of milrinone (PDE3 inhibitor) or vehicle control via intraperitoneal injection. The milrinone group of mice showed no delay in delivery time compared with the LPS control group, but an improvement in pup survival (26.4±7.0% vs. 54.8±11.2%, P<0.05) was observed. This was also accompanied by changes in cytokine relative mRNA levels in myometrium and placenta. These data suggest that PDE3 inhibition may have a neuroprotective effect in our model of PTL. Further studies will establish the mechanisms involved and define whether a combined approach of PDE3 and 4 inhibition is a better combination for neuroprotection and PTL prevention than either alone.

S-078

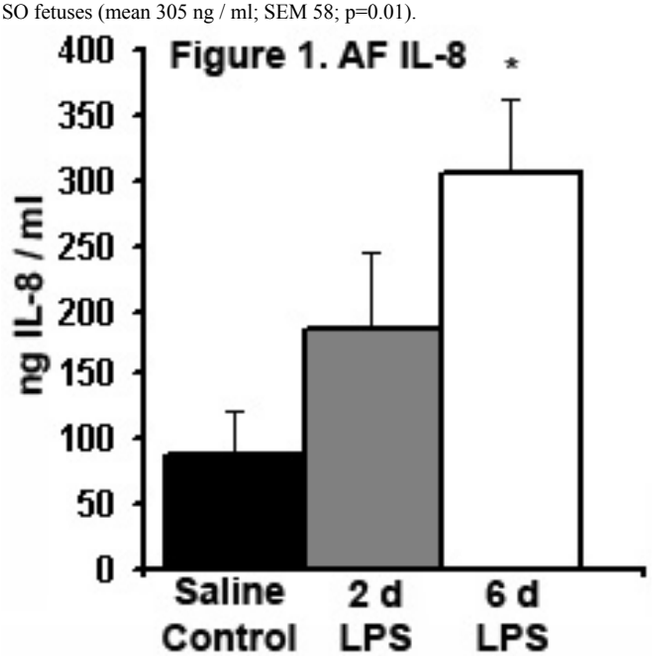
In Situ Assessment of Toll-Like Receptor-4 Expression in Human Endometrial Endothelial Cells (HEEC) during the Menstrual Cycle and Early Pregnancy. Emre Vastandaslar, Nehir Ocak, Frederick Schatz, Charles J Lockwood, Umit A Kayisli. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Context : Toll receptor-4 (TLR-4) mediates host responses against lipopolysaccharide (LPS) and "danger signals" emanating from injured cells. Human endometrium undergoes menstrual cycle and early pregnancy related-inflammation and leukocyte recruitment. Human endometrial endothelial cells (HEEC) directly contact bacteria and inflammatory mediators in the circulation.
Objective: Compare immunoreactive TLR-4 levels in HEEC in endometrial capillaries across the menstrual cycle and in early pregnant decidua.
Methods: Serial sections (5u) from formalin-fixed, paraffin-embedded specimens from normal proliferative phase (n=3) and secretory phase endometrium (n=3) and first trimester decidua (n=3) from elective termination were immunostained for TLR-4. These sections were assessed semi-quantitatively in HEEC by HSCORES.
Results: Comparison of HSCOREs in HEECs indicates weak to moderate immunoreactivity for TLR-4 in proliferative phase endometrium (146.7±14.5; Mean ± SEM). Increased levels of TLR-4 immunoreactivity in the secretory phase (215.7 ± 13.7), that attain significance when compared with proliferative phase HEECs (p<0.05). Like the secretory phase, HEECs in the first trimester also expressed strong TLR-4 immunoreactivity (232.0 ± 17.2), that is also significantly greater that that of the proliferative phase (p<0.05).
Conclusions: Significantly higher immunoreactive TLR-4 levels in HEECs during the secretory phase and early pregnancy suggest that such HEEC are poised to respond to bacteria and inflammatory signals in the local circulation during these phases.

S-079

Inflammation of the Fetal Skin Increases Amniotic Fluid IL-8. Matthew W Kemp,[1] Masatoshi Saito,[1,2] Jeffery A Keelan,[1] John P Newnham,[1] Tom Cox,[1] Alan H Jobe,[1,3] Boris W Kramer,[4] Jennifer G Collins,[4] Shaofu Li,[1] Li Zhang,[1,5] Huixia Yang,[1,5] Suhas G Kallapur.[1,3] *[1]School of Women's and Infants' Health, University of Western Australia, Australia; [2]Division of Perinatal Medicine, Tohoku University Hospital, Japan; [3]Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Centre, USA; [4]Department of Paediatrics, Maastricht University Medical Centre, Netherlands; [5]Health Science Center, Peking University, China.*

Objective
In utero infection and inflammation are causally associated with early preterm birth. We surgically isolated the fetal lung and gut in our ovine model of in utero inflammation to ask if fetal skin inflammation increases cytokine levels in the amniotic fluid (AF).
Methods
Date mated merino ewes were divided into four groups: 1) 2 d snout occlusion (SO) + 10 mg intraamniotic (IA) lipopolysaccharides from E.coli (LPS) (n=6); 2) 6 d SO + IA LPS (n=7) 2) SO + IA saline (n=4 ; 3) sham occlusion + IA saline (n=5). Fetuses were delivered at 124d (term=150 d). AF and fetal skin were analysed for inflammation by qPCR and ELISA.
Results
No change was detected in IL-1β, IL-6, IL-8, TNFα, MCP-1 or IL-10 mRNA in skin from 2 d LPS SO fetuses. 6 d SO + LPS fetuses exhibited significantly increased (fold change) IL-1β (mean 3.00; SEM 0.50; p=0.02), and IL-8 (mean 10.7; SEM 3.4; p=0.01) mRNA expression and no change in IL-6, TNFα, MCP-1 or IL-10 expression. AF IL-8 was significantly increased in 6 d LPS SO fetuses (mean 305 ng / ml; SEM 58; p=0.01).



Conclusions
Elevated fetal skin IL-8 mRNA correlates with significantly increased levels of AF IL-8. We are unable to assess the contribution of the chorioamnion to inflammation in this model; however, we have previously demonstrated a lack of IL-8 mRNA expression in the chorioamnion after 7 d IA LPS exposure (Am J Respir Crit Care Med 2001;164;982) and a lack of intrauterine inflammation following administration of LPS to the subchorionic space (Am J Obstet Gynecol 2003;189:1771). These data provide the first evidence suggesting that fetal skin inflammation makes a significant contribution to elevated levels of a preterm birth-associated cytokine in the AF.

S-080

Differential Response to Lipopolysaccharide by JEG-3 and BeWo Choriocarcinoma Cell Lines. Yong Qin Koh, Marloes Dekker Nitert, Jennifer M Ryan, Kanchan Vaswani, Hsiu-Wen Chan, Murray D Mitchell, Gregory E Rice. *UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia.*

Introduction: Trophoblast cell lines generated from human choriocarcinoma cells (such as JEG-3 and BeWo) have been used to study trophoblast development

and function. Each cell line, however, displays phenotypic differences related to the mutational background of the tissue of origin. Recently, JEG-3 and BeWo cells were used to study the trophoblastic inflammatory response to bacterial lipopolysaccharide (LPS). Here, we provide evidence that these cells differ in protein expression of LPS co-receptor (CD14) and cytokine release.
Objective: To characterise and compare the inflammatory response of JEG-3 and BeWo cells to LPS.
Methods: JEG-3 and BeWo cells were cultured in RPMI 1640 supplemented media. Cells were serum starved overnight and then incubated in the presence of LPS (0-50 μg/mL) for 24-48h. The supernatants were collected and IL6 and TNFα concentrations were determined by ELISA. Cells were harvested for RNA/protein analysis. NFκB (p50 subunit, NM_ 003998) gene and protein expression were determined using real-time PCR and Western blotting, respectively.
Results: Basal CD14 protein expression was approximately 2-fold greater in BeWo cells compared to JEG-3 cells (n=1). Interestingly, IL6 and TNFα release was unaltered in BeWo cells at any concentration and exposure time, but it was significantly upregulated in JEG-3 cells (IL6 secretion after 48h at 50 mg/mL LPS vs control (mean±SD): 198.96±28.74 vs 12.54±5.44 pg/mL, n=3, p<0.05; TNFα after 48h at 50 mg/mL LPS vs control: 28.17±1.55 vs 15.13±2.14 pg/mL, n=3, p<0.05). mRNA expression of NFkB (a transcription factor activated by inflammatory mediators) was also significantly increased after LPS treatment (10 mg/mL) for 24 and 48h in JEG-3 (24h LPS vs control: 1.43±0.15 vs 1.01±0.17, p<0.05; 48h: 1.65±0.22 vs 1.01±0.15, n=3, p<0.05), but not BeWo cells.
Conclusion: JEG-3 cells are responsive to LPS exposure as demonstrated by an increase in NFkB mRNA expression and activation of IL6 and TNFα release. Conversely, BeWo cells are not sensitive to LPS despite apparent higher protein expression of CD14 compared to JEG-3 cells. The data obtained suggest that the JEG3 cell line may be a more suitable model for assessing trophoblast cell inflammatory responsiveness than BeWo cells.

S-081

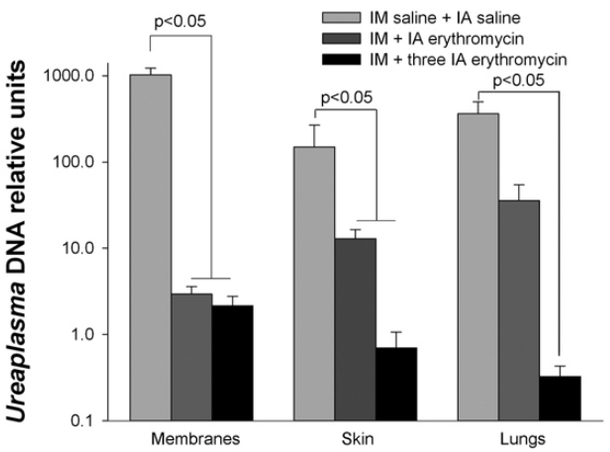
Fastidious Genital Tract Bacteria in the Pregnant Uterus. Sophia R Lannon,[1] Kristina M Adams Waldorf,[1] David N Fredricks,[2] Michael G Gravett.[1] *[1]Obstetrics & Gynecology, University of Washington, Seattle, WA, USA; [2]Vaccine and Infectious Disease, Fred Hutchison Cancer Research Center, Seattle, WA, USA.*
Objective: Recent studies using polymerase chain reaction (PCR) techniques have identified new fastidious bacterial species in women with bacterial vaginosis (BV), a condition associated with preterm birth. The pregnant uterus has been considered to be a sterile environment, but no prior studies have investigated whether these fastidious bacteria colonize the choriodecidua or membranes. We sought to determine whether fastidious bacteria colonize the choriodecidua or chorioamnion in women before or during labor.
Methods: Women at term (≥37 weeks) in labor or not in labor were enrolled, excluding multiple gestations or known infection. Swabs were collected from the vagina, chorioamnion, and choriodecidual space (during cesarean delivery if available). Samples were analyzed by standard laboratory culture, quantitative broad range 16S PCR, and bacterial species-specific quantitative PCR. Vaginal samples were analyzed for bacterial vaginosis using Nugent scoring.
Results: Samples were collected from 24 subjects (11 non-labor, 13 labor). All non-laboring and five laboring subjects delivered by cesarean section. Choriodecidual samples were collected in all non-laboring and two laboring cesarean deliveries. In non-laboring women, no significant bacterial DNA was detected in the chorioamnion and choriodecidua by broad range or species specific PCR. In contrast, 2 laboring women had positive chorioamnion samples (13%). In one of these subjects *Megasphaera*-like bacterium and lactobacillus genus DNA were detected with a corresponding culture of 10^6 colonies/cc Enterococcus and coagulase negative Staphylococcus. The other subject developed clinical chorioamnionitis, and PCR detected *Gardnerella vaginalis, BVAB-2, Leptotrichia/Sneathia, Atopobium vaginae, Megasphaera* and lactobacillus. Corresponding culture grew 10^3 colonies/cc mixed flora including *Gardnerella vaginalis*. Neither subject was positive for BV by Nugent score. Lactobacillus DNA was detected in two additional chorioamnion samples of women with vaginal deliveries.
Conclusions: These results suggest that fastidious vaginal bacteria are not commonly present in the chorioamnion or choriodecidua of non-laboring women, but may occasionally ascend during labor and could play a role in clinical chorioamnionitis.

S-082

The Assessment of the Selected Biochemical Markers in Predicting Preterm Labour. Piotr Laudanski,[1] Grzegorz Raba,[2] Adam Lemancewicz,[1] Pawel Kuc,[1] Rafal Kisielewski,[1] Tadeusz Laudanski.[1] *[1]Department of Perinatology, Medical University of Bialystok, Bialystok, Podlasie, Poland; [2]Department of Obstetrics and Gynecology, Provincial Hospital, Przemysl, Podkarpackie, Poland; [3]Department of Perinatology, Medical University of Bialystok, Bialystok, Podlasie, Poland; [4]Department of Perinatology, Medical University of Bialystok, Bialystok, Podlasie, Poland; [5]Department of Perinatology, Medical University of Bialystok, Bialystok, Podlasie, Poland; [6]Department of Perinatology, Medical University of Bialystok, Bialystok, Podlasie, Poland.*
Objective: The aim of the study was to assess the prognostic importance of the selected markers in predicting preterm labour.
Material and methods:
We have studied 74 patients hospitalized due to threatening preterm labour. 51 women gave birth prematurely; the remaining 23 were diagnosed with false labour. The results have been compared with a reference group of 14 physiological deliveries. We used ELISA arrays to study 20 serum proteins: Acrp30, BLC, b-NGF, GDNF, IL-7, IL-17, M-CSF, IGFBP-1, IGFBP-2, BDNF, L-Selectin, E-Selectin, ICAM-1, PECAM-1, VCAM-1, MIP-1delta, MIP-3alpha, MIP-3beta, Eotaxin-1 and Eotaxin-2.
Results:
The analysis carried out between the three studied groups revealed that an increased risk of preterm labour should be expected when the serum concentration for: IGFBP-1 > 158.83 pg/ml (sens. 0.608, sp. 0.609, p<0.0001); MIP-1delta < 27,66pg/ml (sens. 0.627, sp. 0.627, p=0.021);
BDNF >36,54pg/ml (sens. 0.630, sp. 0.647, p=0,002); BLC >25,46pg/ml (sens. 0.588, sp. 0.609, p<0,001) and Eotaxin-1 >1,16pg/ml (sens. 0.633, sp. 0.652). The significant differences in mean values have been noted for certain biochemical markers when alternative methods were applied, such as: Artificial Neural Network and Discriminant Function Analysis, and the most predictive value was shown for BDNF.
Conclusion:
There have been reported statistically significant differences in serum concentrations of selected proteins in women with preterm labour and false labour. Further studies are required to confirm the results for selected proteins.

S-083

Intra-Amniotic Erythromycin Treatment for Subclinical Intrauterine *Ureaplasma parvum* Infection in Sheep. Shaofu Li,[1] Ilias Nitsos,[1] Jeffrey Keelan,[1] Matthew Kemp,[1] Alan Jobe,[2] Christine Knox,[3] John P Newnham.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, WA, Australia; [2]Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, OH, USA; [3]Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia.*
Objective: Macrolide antibiotics are potentially effective against microorganisms such as *Ureaplasma spp*. (UP), isolated from amniotic fluid of women with preterm birth. However, they have poor access to amniotic/fetal tissues when administered maternally. Our aim was to determine the effectiveness of intraamniotic (IA) erythromycin administration to treat intrauterine UP infection in pregnant sheep. **Methods**: Pregnant ewes with singleton fetuses received IA inoculation of UP serovar 3 at 55 days of gestation (dG). Ewes at 116 dG (n=8-10) were allocated to either 1) maternal intramuscular (IM) saline plus IA saline, 2) maternal IM erythromycin (500 mg/8 h/3 d) plus a single IA erythromycin (3.2 mg/kg fetal weight), or 3) maternal IM plus three IA erythromycin 48 h apart. Amniotic fluid was collected before each injection. Delivery by Caesarean section was at 125 dG. Genomic DNA was extracted from amniotic fluid and fetal membranes, lungs and skin. Quantitation of UP DNA was performed by qPCR. **Results**: Amniotic fluids from all UP-inoculated animals at 116 dG were positive for UP DNA. The amount of UP DNA in membranes and skin was significantly reduced compared to controls (p<0.05, Kruskal Wallis) following IM administration of erythromycin with either a single IA or repeated IA dose at 125 dG. However, in the lungs repeated IA erythromycin treatment was significantly more effective than a single IA dose.



Conclusions: IM/IA erythromycin is effective in reducing levels of UP in intrauterine tissues. Repeated IA administration of erythromycin had additional tissue-specific benefits compared to a single IA dose. Adjunctive IA erythromycin treatment of intrauterine UP infection may be an effective modality for the prevention and/or treatment of preterm delivery, although fetal clearance has yet to be shown.

S-084

Effect of *Lactobacillus rhamnosus* GR-1 (GR-1) on Lipopolysaccharide (LPS)-Induced Cytokine Production Profiles from Human Term Decidual Cells. W Li,[1] S Yang,[1] SO Kim,[2] G Reid,[2] JR Challis,[1] AD Bocking.[1] *[1]Department of Obstetrics & Gynecology, University of Toronto; [2]Department of Microbiology and Immunology, University of Western Ontario.*

Objective: Probiotics are of increasing interest as adjuncts to prevent and possibly treat infection-induced preterm birth. Decidua is the functional layer of the endometrium of pregnancy, containing a variety of immune cells. This study aimed to investigate the effect of *L. rhamnosus* GR-1 on LPS-induced profiles of cytokine and chemokine production in term human decidual cells (DC). **Methods**: DC were isolated by collagenase from human term fetal membranes and cultured with estradiol (10^{-9}M)/progesterone (10^{-9}M) and treated with or without LPS (10ng/ml) and /or GR-1 supernatant (1:20) for 8h. Cytokines in culture medium were analyzed by Bio-Plex Human Cytokine 27-plex assay and ELISA. Results: LPS significantly increased pro-inflammatory cytokines [interleukin-1 (IL-1), IL-2, IL-12, IL-17, IFN$_\gamma$ and TNF$_\alpha$], anti-inflammatory cytokine [IL-1ra, IL-4, IL-9, IL-10, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage-CSF] and chemokine [IL-8, eotaxin, interferon-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), regulated on activation normal T cell expressed (RANTES)] output with a range from 1.4 to 44 fold ($p<0.05$) . The composite ratio of pro-inflammatory cytokines to anti-inflammatory cytokines was significantly increased by LPS, and in particular the ratio of TNF$_\alpha$ to anti-inflammatory cytokines from 0.67 (control) to 4.75 (LPS). GR-1 alone significantly increased G-CSF and MIP-1$_\beta$, but decreased IP-10 output ($p<0.05$). GR-1 also significantly reduced all LPS-induced cytokine output ($p<0.05$) except for G-CSF, MIP-1$_\alpha$, MIP-1$_\beta$, MCP-1, IL-8 and IL-9. The composite ratio of pro-inflammatory cytokine to anti-inflammatory cytokine was also significantly decreased by GR-1, including the ratio of TNF$_\alpha$ to anti-inflammatory cytokines from 4.75 (LPS) to 0.59 (LPS+GR-1). **Conclusion**: LPS induces a shift in pro-inflammatory bias and GR-1 redirects the balance by decreasing LPS-induced pro-inflammatory cytokine production and increasing anti-inflammatory cytokine productionin in cultured human decidual cells. GR-1 also increases chemokine production . We speculate that *L. rhamnosus* GR-1 may inhibit bacteria-induced inflammatory responses and activate phagocytes, thereby limiting inflammation and enhancing pathogen clearance.

S-085

***Escherichia coli* Lipopolysaccharides Induce Serotype-Specific Responses in Murine Models of Pre-Term Labour.** David A MacIntyre,[1] Bronwen Herbert,[1] Yun S Lee,[1] Mark R Johnson,[2] Phillip R Bennett.[1] *[1]Surgery and Cancer, Imperial College London, London, United Kingdom; [2]Obstetrics and Gynaecology, Chelsea and Westminster Hospital.*

Lipopolysaccharide (LPS) is a component of the outer cell membrane of gram-negative bacteria that is used to stimulate inflammatory pathways in animal models of infection/inflammation-induced pre-term labour (PTL). Apart from different methods of administration, various forms of LPS are often utilised, which may account for some of the inconsistencies reported between models. LPS is composed of a complex glycolipid made up of Lipid A (a phosphorylated glucosamine disaccharide with multiple fatty acids chains that is generally conserved among gram-negative bacteria), an oligosaccharide core and an extending gylcan polymer known as the O-antigen. The sugar composition of this polysaccharide side chain determines the serological specificity of the molecule. Moreover, studies in rat models of hypothermia and albumin extravasation have reported marked functional differences caused by LPS serotype specificity[1,2]. In consideration of these findings, we aimed to determine if LPS-serotype specific responses exist in a murine model of infection/inflammation-induced PTL. Timed pregnant CD-1 mice were administered at E16 with a 20 µg intrauterine injection of either *Escherichia coli* LPS serotype O111:B4 (n=6), O55:B5 (n=5), O127:B8 (n=5), O128:B12 (n=5) or PBS control (n=18). All LPS types were subjected to phenol extraction. While PBS control dams delivered approximately 58 h post injection (p.i., SEM ± 3h), 0111:B4 injected animals delivered 7 h p.i. (± 1), O55:B5 delivered 10 h p.i. (± 1), O127:B8 delivered 16 h p.i. (± 4) and O128:B12 at 17 h p.i. (± 1). Pup survival rates at 6 h were 100% for PBS and O128:B12, however, in the case of O111:B4, all pups were dead at 6h. Pup survival rates for O55:B5 and O127:B8 serotypes were 80% and 95% respectively. Our findings suggest that LPS induces serotype-specific responses in murine models of pre-term labour that effect both maternal and fetal response mechanisms. We propose that this is due to the LPS serotype-specific activation of distinct inflammatory pathways.

[1]Eyup et al., Am J Physiol Regul Comp Physiol 292: R1846-R1850, 2007
[2]NedrebØ et al., Shock 18,2:138-141, 2002

S-086

Racial Disparity in *Trp53* Gene Mutations and Their Association with Spontaneous Preterm Birth. Emily DeFranco,[1] Ramkumar Menon,[2] Ge Zhang,[3] Wei Ang,[4] SK Dey.[3] *[1]OB/GYN, University of Cincinnati, Cincinnati, OH; [2]OB/GYN, UTMB, Galveston, TX; [3]Divisions of Human Genetics and Reproductive Sciences, Cincinnati Children's Hospital, Cincinnati, OH; [4]School of Women's and Infants' Health, University of Western Australia, Perth, Australia.*

Objective: The tumor suppressor protein p53, encoded by *Trp53*, is important for maintaining genomic stability. Polymorphisms in *Trp53* gene have been associated with early pregnancy failure in mice and humans. We tested the association of rs2287499 (Arg68Gly) and rs1042522 (Pro72Arg), two common single nucleotide polymorphism (SNP) variations in *Trp53* that show protein alteration in humans, with the outcomes of spontaneous preterm birth with intact membranes (sPTB) and preterm premature rupture of the membranes (pPROM). We also assessed the racial disparity in SNP frequency between African American (AA) and Caucasian (C) parturients.

Methods: In a case (pPROM [AA=34;C=49]; PTB [AA=108;C=245])-control (term birth;AA=338;C=655)study, DNAs from maternal blood were collected from a population in Nashville, TN, USA. Genotyping *Trp53* SNPs (rs2287499 and rs1042522) was performed using TaqMan assay kit. Differences in SNP frequencies between races and their independent association with sPTB and pPROM were analyzed. Race was self reported back to three generations from both maternal and paternal side.

Results: Genotypic frequencies of the SNPs differed between AA and C in both cases and controls ($p<0.0001$). In stratified analyses neither SNP was associated with sPTB ($p=0.9$ and 0.2) or pPROM ($p=0.3$ and 0.5) in C. In AA, no association between rs1042522 and sPTB ($p=0.7$) or pPROM ($p=0.8$) was found but there was a marginally significant association between rs2287499 AA sPTB($p=0.09$) but not with pPROM($p=0.5$).

Conclusion: Racial disparity exists in the genotypic frequencies of the two SNPs tested. These SNPs are not associated with sPTB or pPROM in C, which may be partly attributed to the complexity of the phenotype. The marginal significance in AA sPTB with rs2287499 is worth replicating in larger sample size as AA sPTB is more commonly associated with infection/inflammation and cell death where p53 plays a major role. These associations could represent genetic predisposition to environmental insults leading to sPTB and pPROM. Replication in larger populations and analysis of other *Trp53* SNPs that are not in linkage disequilibrium with those studied will better inform the role of genetic variations in *Trp53* and preterm birth.

S-087

Amniotic Fluid Antimicrobials and Antioxidants Show Racial Disparity in Preterm Birth. Ramkumar Menon,[1] Tomi Kanninen,[3] Catherine Herway,[3] George Saade,[1] Stephen J Fortunato,[2] Steven S Witkin.[3] *[1]Ob & Gyn, The University of Texas Medical Branch; [2]The Perinatal Research Center, Centennial Women's Hospital; [3]Ob & Gyn, Cornell University.*

OBJECTIVE: Intraamniotic infection and oxidative stress are two major pathophysiologies associated with spontaneous preterm birth (PTB). Compromises to the antimicrobial and redox environment in the amniotic cavity would promote intraamniotic microbial invasion. We measured two recently identified amniotic fluid (AF) antimicrobials, Hyaluronan (HA), and Histone 2B (H2B), and the antioxidant, superoxide dismutase (SOD), in African American (AA) and Caucasian (C) women with preterm and term births. We hypothesized that profile differences may indicate racial disparity in the rate of infection-associated PTB.

METHODS: In a case (PTB with intact membranes < 37 weeks; AA = 22, C = 31) control (normal term birth > 37 weeks; AA = 37, C = 42) study AF samples were collected by transvaginal amniocentesis during labor at preterm or term. Concentrations of HA, H2B and SOD were measured by ELISA. Statistical analysis compared differences in concentration between cases and control between races. Stratified analysis among cases based on histologic chorioamnionitis (HC) status was also performed.

RESULTS: In a combined analysis, no significant differences in the median concentrations of HA, H2B and SOD were seen between cases and controls. However, compared to their respective controls AA cases had lower HA (519 pg/ml vs. 665 pg/ml; p=0.03) and higher H2B (252 ng/ml vs. 166 ng/ml; p=0.05) and SOD (1.2 U/ml vs. 0.8 U/ml; p = 0.006). In contrast, analyte concentrations were not significantly different between C cases and controls. In addition, AA samples at term had significantly lower concentrations of H2B and SOD compared to C at term. Stratified analysis based on presence (n=9) or absence (n=44) of HC showed higher concentrations of H2B (p = 0.02) and SOD (p=0.04) in cases with HC.

CONCLUSION: We report racial disparity in AF antimicrobial and antioxidant factors. Dysregulated AF production of HA, H2B and SOD was associated with PTB in AA but not in C supportING an underlying immune and redox imbalance as contributing to PTB in the former group. This is consistent with our prior reports suggesting an overwhelming inflammatory response in AA PTB whereas inflammation is likely a secondary phenomenon in C PTB.Race should be considered as a risk modifier and stratified analysis is warranted with PTB biomarker data.

S-088

Occult Infections in Placenta as an Etiology for Preterm Birth. Carolyn Bower, Rebecca Gunkel, Bridget Conlon, Michelle Landeau, Kim Delli-Zotti, Quihong Zhao, Fredrick Kraus, Donald Nelson, George Macones, Indira Mysorekar. *Obstetrics & Gynecology, Washington University School of Medicine, St. Louis, MO, USA.*

Prematurity is a leading cause of perinatal morbidity and mortality in developed countries. A frequent and important mechanism underlying prematurity is intrauterine inflammation and infection, yet how these initial insults might lead to preterm labour (PTL) and preterm birth (PTB) is poorly understood. Intriguingly, women with a previous PTB have up to a 5–fold increased risk of PTB in subsequent pregnancies, suggesting a pre-pregnancy maternal endometrial infectious etiology for recurrent PTB. However, little is known about the cellular and physiological mechanisms underlying recurrent PTB. We posit the novel concept that persistent and clinically occult infections, caused by organisms that form biofilms harboured within uterine tissues and inducing inflammatory cascades, represent one etiology of PTB. Our objective was to substantiate the concept that persistent and clinically occult infections may serve as one cause of increased risk for repetitive preterm births. We accrued pregnant women presenting with premature labour (n=55) and our control group included women who delivered at 37-40 weeks gestation (n=75) without serious medical complications, or antibiotic treatment. Placental tissues from all enrolled PTB and control patients were analyzed in systematic random sampling of the maternal basal plate tissue (n=3 samples/tissue) and stained for presence of intracellular microorganisms. Each decidua basalis specimen was examined in 10 random fields and quantified for the presence and location of microorganisms per 100 micron^2**.** Excitingly, we identified intracellular bacteria in 29% of decidual tissues from PTB placentas compared with 10% among term. Our findings suggest a strong relationship between presence of intracellular infection and prematurity and that occult reservoirs of micro-organisms in the maternal basal plate may serve as one etiology for PTB.

S-089

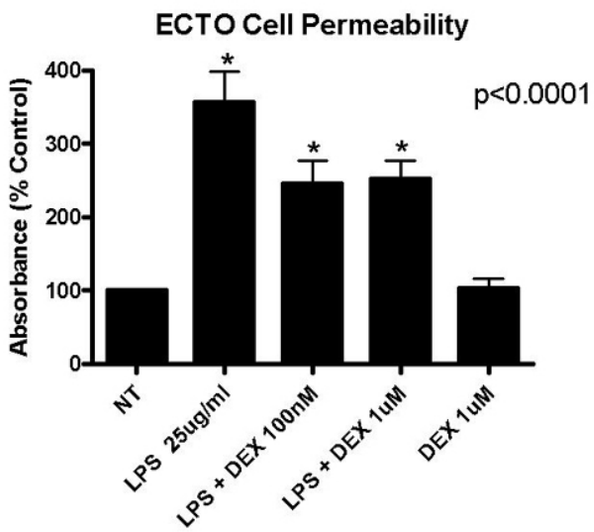
Inflammation Provoking a Cytokine Response and Disrupting the Epithelial Barrier Is Mitigated with Dexamethasone: A Possible Mechanism of Premature Cervical Remodeling and Preterm Birth. Christopher Nold, Lauren Anton, Brown Amy, Michal Elovitz. *Obstetrics and Gynecology, University of Pennsylvania Perelman School of Medicine, Maternal and Child Health Research Program, Philadelphia, PA, USA.*

OBJECTIVE: Premature cervical remodeling is likely an obligatory step in the pathogenesis of spontaneous preterm birth (PTB). We have recently demonstrated that an inflammatory challenge can disrupt the cervical epithelial barrier. These studies sought to assess if dexamethasone (DEX) could prevent disruption of the epithelial barrier from an inflammatory stimuli.

STUDY DESIGN: Immortalized ectocervical (ECTO) and endocervical (ENDO) cells were used for this study. The cells were plated at a concentration of 9.6 x 10^4 per well for 24 hours. Cells were treated with 100nM or 1uM of DEX for 2 hours prior to treatment with 25ug/ml of lipopolysaccharide (LPS). Media was collected at 6 and 24 hours. The cytokines IL6 and IL8 were assessed at both time points. A measure of epithelial disruption, soluble E-Cadherin (SECAD), was also measured. The effect of LPS, DEX or LPS + DEX on the integrity of the epithelial cell barrier was assessed using an in vitro permeability assay.

RESULTS: Both IL6 and IL8 levels were significantly decreased at 6 hours (P<0.01) in ECTO and ENDO cells treated with DEX+ LPS compared to LPS alone. IL6 levels were also decreased at 24 hrs (P<0.05) in DEX+LPS compared to LPS alone in ECTO but not ENDO cells. In ENDO cells, DEX also limited IL8 response at 24 hrs (P<0.01). Pretreatment with DEX did not prevent the LPS-induced rise in SECAD. DEX did prevent the LPS-induced change in permeability in ECTO cells (p<0.0001) (FIG 1).

CONCLUSION: These studies demonstrate that DEX can decrease the cytokine response in cervical epithelial cells from an inflammatory challenge as well as limit the inflammation-induced change in the epithelial barrier. These results suggest that anti-inflammatory therapies may be able to limit premature cervical remodeling and hence be a possible therapeutic strategy to reduce prematurity.



S-090

Immune Adaptations of Pregnancy as Risk Factors of Gestational UTI. Bogdan J Nowicki, Stella Nowicki. *Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*

PURPOSE: ABU/UTI/pyelonephritis affecting a mother during gestation may have a life-long impact on both mother and the fetus. Until now aggressive screening and antibiotic therapy to prevent UTI is our best option. We previously reported data on temporary patterns showing a trimester-specific predominance of *E. coli* serotypes, *E. coli* DNA fingerprints and E. coli bearing Dr adhesins. Further, in recent years, there has been an accumulation of research data on the altered immune responsiveness in pregnant animals with experimental UTI and/or with uterine infections. Here we present an update on those immune/risks factors in pregnant patients with UTI.

METHODS: We propose that in pregnant mothers in whom nitric oxide (NO) is operating at a maximum or near-maximum capacity and therefore has limited capacity to increase in response to a spreading infection, and in whom TLR4

is down-regulated, the simultaneous limited/diminished responsiveness of both NO and TLR4 plays a key role in a reduced gestational responsiveness to inflammation and infection. This may allow ABU to progress to UTI and urogenital infections or even fatal septicemia. Therefore in an environment in which two key protective systems, NO and TLR4, have limited or a decreased capacity to display their functions and in which the third factor E. coli receptor CD55 is elevated, we observe a paradoxical increase in Dr+ *E. coli*, which in turn finds a niche of opportunity to establish chronic and/or subclinical infections. In the context of the immune adaptations, the infection process caused by Dr+ *E. coli* may appear uniquely tailored to the environment of pregnancy with gestational tropism/virulence mediated by Dr adhesin.

CONCLUSION:A combination of Dr *E. coli* gestational tropism in the presence of NO/TLR4 and CD55 adaptations and E. coli antibiotic resistance poses the question: should we apply a novel molecular approach to diagnose high risk pregnancy-associated pathogen/virulence factors and in addition, specifically tailor antibiotic therapy standards to improve maternal and fetal safety?

S-091

Appropriate for Gestational Age-Preterm Neonates Born to Mothers with Moderate, but Not Mild or Severe, Intensity of Histologic Chorioamnionitis, Funisitis, and Intraamniotic Inflammation without Microbial Invasion of Amniotic Cavity Have a Lower Rate of RDS. Chan-Wook Park, Bo Hyun Yoon, Joong Shin Park, Jong Kwan Jun. *Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Korea.*

Objective: RDS is reduced by exposure of intrauterine inflammation(IUI) and "spontaneous"causes of preterm birth. However, our recent study(SGI2012 abstract #651035) reported that only SGA is related to a lower rate of RDS in preterm newbonrs(NB) born to mothers even without IUI.A fundamental question is whether various degrees of IUI have a different effect on development of RDS in AGA preterm newborns due to"spontaneous"causes. The purpose of this study was to answer this issue.

Methods: A cohort study was conducted and included 378consecutive singleton AGA(>5th percentile for GA) newborns<34wks due to preterm labor or preterm PROM. MIAC and IAI were assessed in 196AF obtained within 7days of birth. AF was cultured for aerobic/anaerobic bacteria and genital mycoplasmas.IAI was defined as an AF MMP8>23ng/ml and divided into 4 groups(Gr) according to AF MMP8 level(ng/ml):1) mild,23~100;2) moderate,100~500;3) moderate, 500~1000;4)severe, 1000~. Histologic grade (gr) in HCA was defined by the criteria past published(*AJOG*1995;172:960)(total gr:1~8).The analysis for differences in rate of RDS between cases with sterile status and various gr of HCA, funisitis and IAI with or without MIAC were adjusted for GA at delivery,antenatal steroid use and C/S.

Results:1)In contrast to HCA gr2~3 or 4~5(each for p<.05),HCA gr1 or 6~8 was not related to a lower rate of RDS than sterile placenta (each for p>.05);2) Although funisits(20%, p<.005) reduced the rate of RDS, funisitis with gr>5(33%, p>.05), but not gr<6(14%, p<.05), didn't reduce the rate of RDS;3) As compared with moderate IAI(Gr2 and Gr3; each for p<.05), neonates born to mothers with mild or severe IAI didn't have a lower rate of RDS than those without IAI(Gr1 or Gr4;each for p>.05) in cases without MIAC;4)However,as the more intense IAI from mild to severe, the more gradually increased rate of RDS(IAI(-): 0%,Gr1:13%,Gr2: 20%,Gr3: 27%,Gr4: 40%; p<.05,Chi square for trend) in the context of MIAC.

Conclusion:AGA preterm NB born to mothers with moderate,but not mild or severe, intensity of HCA, funisitis and IAI without MIAC had a lower rate of RDS. These novel findings suggest that specific intensity of IUI,but not inflammation itself, has a profound effect on fetal lung maturation.

S-092

Human β Defensin -3 in Human Pregnancy: The Effect of Gestational Age, Parturition, and Intra-Amniotic Infection/Inflammation. Manasi Patwardhan,[1,2] Tinnakorn Chaiworapongsa,[1,2] Zeynep Alpay Savasan,[1,2] Edgar Hernandez-Andrade,[1,2] Hyunyoung Ahn,[1] Lami Yeo,[1,2] Zhong Dong,[1] Sonia S Hassan,[1,2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective: Human β Defensin -3 (HBD-3) is an antimicrobial peptide involved in the innate immune response and has a broad range of antimicrobial activity against *E.Coli* and *Staphylococcus aureus*. The purpose of this study was to determine whether HBD-3 is present in the amniotic fluid (AF) and its concentration changes as a function of gestational age, during labor, and in intraamniotic infection/inflammation (IAI).

Methods: AF samples were collected from patients in the following groups: 1) midtrimester (MT) who delivered at term (n=43); 2) term not in labor (n=19); 3) term in labor (n=47); 4) preterm labor with intact membranes (PTL) without IAI (n=52); and 5) PTL with IAI (n=41). IAI was defined as either a positive AF culture or AF interleukin (IL)-6 ≥2.6 ng/mL. AF HBD-3 concentrations were determined by ELISA.

Results: 1) AF HBD-3 was detectable in 98% (197/202) of the samples; 2) patients with PTL with IAI had a median AF HBD-3 concentration significantly higher than those without IAI (IAI: median 11.2 ng/ml, interquartile range (IQR) 5.8-17.6 ng/mL vs. without IAI: median 5.1 ng/ml, IQR 2.6- 9.0 ng/mL; p<0.001); 3) among patients with PTL without IAI, there was no significant difference in the median AF HBD-3 concentration between those who delivered preterm and those who delivered at term (delivered preterm: median 8.6 ng/ml, IQR 2.8-11.5 ng/mL vs. delivered at term: median 4.09 ng/ml, IQR 2.2 – 7.0 ; p>0.05); 4) women at term in labor had a significantly higher median AF HBD-3 concentration than those without labor (labor: median 4.2ng/ml, IQR 2.8-5.9 ng/mL vs. no labor: median 1.9 ng/ml, IQR 0.9- 2.5 ng/mL; p=0.001); and 5) there was no significant difference in the median AF HBD-3 concentration between MT and term no labor (MT: median 2.2ng/ml, IQR 1.6-2.7 ng/mL vs. term no labor: median 1.9 ng/ml, IQR 0.9-2.5 ng/ml; p>0.05).

Conclusion: 1) HBD-3 is present in human AF; 2) IAI is associated with an increased AF HBD-3 concentration, suggesting that HBD-3 is part of the host inflammatory response; and 3) parturition at term is associated with an increase in AF HBD-3 concentration.

S-093

Chronic Pyelonephritis in Pregnancy: Intracellular Survival & Persistance of the Gestational Pathogen Dr+ *E. coli*. Tanu Rana,[1] Rajbir Singh,[2] Stella Nowicki,[2] Bogdan Nowicki.[1] *[1]Obsterics and Gynecology, Meharry Medical College, Nashville, TN, USA; [2]Microbiology and Immunology, Meharry Medical College, Nashville, TN, USA.*

PURPOSE: *E. coli* bearing Dr adhesins (Dr+ *E. coli*) may cause chronic/recurrent pyelonephritis in pregnant women and in experimental rodents. Dr+ *E. coli* binds to complement inhibitor decay-accelerating factor (DAF/CD55), invade and persist in epithelial cells. We postulate that Dr+ *E. coli* capacity to invade and persist contributes to chronic gestational infections. In the present study, we attempt to identify the DAF residues which facilitate invasion, intracellular survival and persistence of Dr+ *E. coli.* **METHODS:** Specific amino acids in the SCR-3 domain of DAF were mutated to alanine and were subjected to gentamicin protection/survival assay in CHO cells, TEM and 3-D structure analysis were done to explain their role in the invasion and survival of Dr$^+$ *E. coli.* Confocal microscopy and live imaging were done to delineate the process of intracellular survival. **RESULTS:** DAF mutants were divided into four groups based on their ability to influence binding and internalization of Dr$^+$ *E. coli* into the CHO-DAF cells. Substitution at Ser165 residue decreased internalization and intracellular survival of Dr$^+$ *E. coli* by 60%. Although replacement of Phe154 with Ala did not affect invasion (100% control), however time course of intracellular survival showed rapid increase of CFUs, 500 at 30 min, 1200 at 60 min while there was no change in CFUs in the control CHO-DAF+, they were 100 at 30 min and 98 at 60min. We observed a chronic (6 days) intracellular persistence of Dr+ *E. coli* without evidence of epithelial cell death and bacterial multiplication in HeLa cells. Confocal microscopy and live imaging revealed that Dr+ *E. coli* prevent the phago-lysosomal fusion and hence intracellular survive. **CONCLUSION:** Dr+ *E. coli* can survive and persist in epithelial cells by escaping the phagolysosomal fusion. Replacement of amino acid Phe154 with Ala facilitated an unexpected increase of intracellular multiplication of Dr+ *E. coli.* This study suggests that Dr+ *E. coli* have evolved to exploit DAF for a non destructive parasitism allowing long term intracellular survival. The intracellular survival and persistence of this gestational pathogen may explain the chronic pyelonephritis caused during pregnancy. Supported by HD41687 from the NICHD to S.N. and by DK42029 from the NIDDK to B.J. N and by U54 RR026140 (NCRR).

S-094

Differential Cytokine and Tissue Remodeling Responses in Chorion and Decidua Exposed to *Ureaplasma parvum*. CE Ransom,[1] PC Seed,[1] KB Fortner,[2] L Feng,[1] L Lan,[3] M Yu,[3] AP Murtha.[1] *[1]Ob/Gyn, Duke University; [2]Ob/Gyn, Vanderbilt University; [3]Biostatistics, Duke University.*

Objective: *Ureaplasma species* have been linked to preterm premature rupture of membranes (PPROM) and preterm birth. We have previously demonstrated bacterial localization adjacent to the membrane rupture site in PPROM subjects (most commonly *Ureaplasma* spp. and *Mycoplasma spp.*), and have shown

adherence of *Ureaplasma parvum* (UP) to primary cells in culture originating from fetal membranes. Our objective was to examine the specific inflammatory response of UP exposure in amnion, chorion and decidua cells isolated from fetal membranes.

Methods: Primary cells of the amnion, chorion and decidua from elective, term cesarean deliveries without labor or PPROM were isolated and confirmed to be free of bacteria, including *Mycoplasma spp.* and *Ureaplasma spp.*. To investigate the inflammatory response to UP, the cells were exposed to UP, E. Coli (EC), or media alone, and inflammatory and tissue remodeling factors were measured from the culture supernatants by Luminex assay. Factorial ANOVA was used to examine differences between exposed cell types. The Type 1 error was adjusted to 0.005 to adjust for multiple comparisons.

Results: Significant differences were measured in IL1b, IL1ra, IL4, Il8, TNF-α and MMP10 in culture supernatants from UP-exposed chorion and in IL1b, IL4, TNF- α and MMP2 from UP-exposed decidua compared to uninfected controls. No significant cytokine response was observed in UP-exposed amnion compared to control. Significant differences were measured in IL1b, IL1ra, Il4, IL6, IL7, IL8, IL9, IL10, IL15, GMCSF, IFNg, MIP1a, MIP1b, TNF-α, and MMP2 in culture supernatants from EC-exposed decidua; no significant cytokine response was observed in EC-exposed amnion or chorion compared to control. In addition, an ANOVA model for all biomarkers found significant differences between amnion/chorion (fetal) cells versus decidua (maternal) cells for IL1b, IL1ra, IL4, IL8, GCSF, IFN-g, TNF- α, MMP2, and MMP10.

Discussion: This study provides evidence that UP exposure produces cell-specific inflammatory cytokine and tissue remodeling responses in primary chorion and decidua cells isolated from fetal membranes, which is distinct from EC. Such changes may promote cell death and loss of membrane integrity, resulting in PPROM. Furthermore, differences in tissue response to bacterial challenge were observed between fetal (amnion & chorion) and maternal (decidua) cell types.

S-095

Disabled Cervical Cytokine Expression in Post-Term Women with Failed Labor Induction. Nathalie Roos,[1] Eva Andersson,[1] Olof Stephansson,[2] Lena Sahlin,[3] Ylva Stjernholm-Vladic,[1] Gunvor Ekman-Ordeberg.[1] *[1]Dep. of Women's and Children's HealthDiv of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden; [2]Clinical Epidemiology Unit, Dep. of Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Dep. of Women's and Children's Health, Div for Reproductive Endocrinology, Karolinska Institutet, Stockholm, Sweden.*

Objective: To study cervical pro- and anti-inflammatory cytokine expression and localization in post-term women with failed and successful labor induction.

Background: Not all post-term women respond to locally administered prostaglandins why delivery is carried out with cesarean section. The ratio between pro- (IL-8) and anti-inflammatory (IL-10) cytokines increases with gestation. Cervical ripening is associated with leukocyte infiltration and increase of IL-8 while failed labor induction post-term is characterized by impaired leukocyte migration into the cervix. Cytokine balance can hence be considered to be involved in recruiting leukocytes and remodeling of cervical extracellular matrix (ECM) at term. We hypothesize an impairment in the shift of ratio of pro- and anti-inflammatory cytokines in post-term women failing to respond to prostaglandins. **Methods:** Cervical biopsies from post-term women were collected: responders (R; n=14) with successful labor inductions and non-responders (NR; n=12) with failed labor induction after prostaglandin E2 priming and term controls (C; n=21) with spontaneous deliveries. Biopsies were taken and analyzed for cytokines IL-8 and IL-10 by real-time PCR and immunohistochemistry. **Results:** The mRNA levels of IL-8 and IL-10 were significantly lower in NR as compared to C and R. In squamous epithelium (SQ) the immunoreactivity for IL8 and IL10 was significantly stronger in NR and R as compared to C and no significant differences between the groups for glandular epithelium (GE) and endothelium (E). For IL-8 there was a stronger immunoreactivity in ECM in R as compared to C and NR. For IL-10 there was a significantly higher immunoreactivity for leukocytes in R as compared to C and NR. **Conclusion:** There is an overall down-regulation of pro- and anti-inflammatory cytokines in cervical tissue from post-term non-responders. The regulation at protein level is complex and differs between cell types. Failed cervical ripening is associated with lower expression of IL-8 and IL-10 in cervical ECM possibly secondary to disabled leukocyte migration or could be the primary to impaired cervical leukocyte influx.

S-096

Inflammation of the Fetal Skin Following Low-Grade Microbial Stimulation. Masatoshi Saito,[1,2] Nobuo Yaegashi,[1] Junichi Sugawra,[1] Tadashi Matsuda,[1] Thomas Cox,[2] John P Newnham,[2] Matthew W Kemp.[2] *[1]Division of Perinatal Medicine, Tohoku University Hospital, Sendai, Japan; [2]School of Women's and Infants' Health, The University of Western Australia, Perth, Australia.*

Objective

Despite being causally associated with early preterm birth (<32 weeks), the magnitude of microbial stimulation required to initiate an acute inflammatory response in utero remains unclear. We employed an ovine model of in utero inflammation to assess the ability of the fetal skin to respond to low-grade stimulation with microbial agonist.

Methods

Date mated merino ewes were randomised to four groups (n=5-7): 1) intraamniotic injection (IAI) of 2 ml sterile saline (control); 2) IAI of 1 mg O55:B5 E.coli lipopolysaccharides (LPS) in 2 ml saline; 3) IAI of 2 mg LPS in 2 ml saline; 4) IAI of 4 mg LPS in 2 ml sterile saline. Fetuses were delivered 2 d post-injection under terminal maternal anaesthesia. Skin from the inner thigh was snap frozen in liquid nitrogen for cytokine analysis by qPCR. One-way ANOVA / Tukey's test were used to assess significance of apparent differences in normalised dCq values across groups.

Results

Relative to control animals, skin from animals exposed to either 1 mg, 2 mg or 4 mg IAI LPS significantly (p=0.010) increased (fold change) IL-8 (mean 4.0, SEM 0.98; mean 4.8, SEM 1.90; mean 4.5, SEM 1.25; respectively) mRNA expression (Figure 1). Relative to control, expression of IL-1β, IL-6, TNFα, MCP-1 and IL-10 mRNA was unchanged in all LPS exposed groups (p>0.05; data not shown).

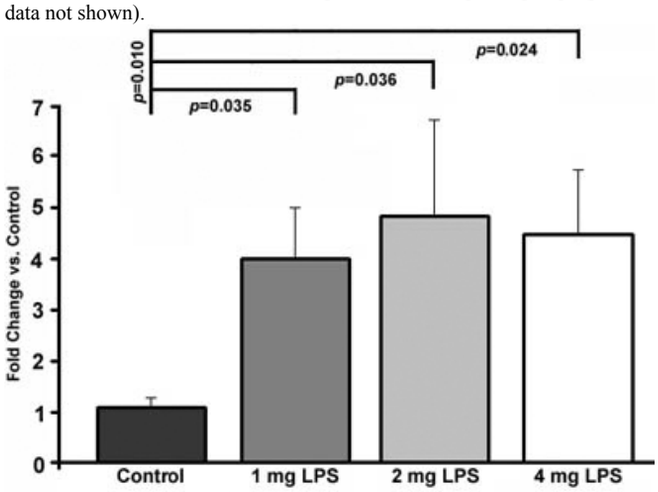


Figure 1. *IL-8* expression in the fetal skin

Conclusions

Preventing infection-driven preterm birth requires understanding the nature and origins of in utero inflammation. The fetal skin initiates an acute inflammatory response to the presence of inflammatory agonist at an IAI dose at least 10 times lower than initially reported. These novel data further support the importance of the fetal skin in the generation of in utero inflammation causally linked to early preterm birth given: 1) the significant proportion of fetal mass comprised by the skin; 2) the fetal skin's exposure to the amniotic environment; and 3) the association of low-grade, chronic infection with early preterm birth.

S-097

Immunoreactive Toll-Like Receptor 4 Expression in Decidual Cells Versus Trophoblasts in the Decidua across Pregnancy. Frederick Schatz, Umit A Kayisli, Nehir Ocak, Emre Vatandaslar, Graciela Krikun, Charles Lockwood. *Obstetrics, Gynecology & Reproductive sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background: Toll receptor-4 (TLR-4) mediates host responses against gram negative bacteria-derived lipopolysaccharide (LPS) and "danger signals" emanating from injured or dying cells accompanying excess inflammation even in the absence of infection. Decidual cells and trophoblast are in close contact in the decidua across human pregnancy. Unlike extensive studies of TLR-4 expression in placental trophoblast, there are few studies in decidual cells.

Objective: In specimens matched for maternal age, we compared immunoreactive TLR-4 levels in decidual cells and trophoblast in sections from first and second trimester decidua basalis and third trimester decidua basalis and parietalis.
Methods: Serial sections (5um) were obtained from formalin-fixed, paraffin-embedded specimens from first (n=5) and second trimester (n=4) elective terminations and third trimester (n=4) normal deliveries. Sections were immunostained for TLR-4 and cytokeratin, a trophoblast marker, and vimentin, a decidual cell marker. TLR-4 immunohistochemical (IHC) staining was assessed semi-quantitatively in decidual cells and trophoblasts by HSCORE.
Results: Cell membranes and cytoplasm of decidual cells and interstitial trophoblast displayed immunoreactive TLR-4. In decidual cells: TLR-4 HSCORES were significantly higher in first and third trimester versus second trimester, whereas in the third trimester TLR-4 HSCORES were similar in decidual cells present in decidua parietalis and basalis. In interstitial trophoblasts: TLR-4 HSCOREs were significantly higher in first than second trimester ($p<0.01$). Across pregnancy, TLR-4 HSCORES were higher in decidual cells than in interstitial trophoblast at every site examined with statistical significance attained in the third trimester ($p<0.01$). Throughout pregnancy, syncytiotrophoblasts displayed consistently weak TLR-4 immunoreactivity.
Conclusions: Higher immunoreactive TLR-4 levels in decidual cells than trophoblast across gestation point to decidual cells as the primary target for LPS derived from gram negative bacteria and/or inflammation-related danger signals in the presence or absence of infection.

S-098

Priming of Human Decidual Cells with a TLR-7 Agonist Suppresses Tumor Necrosis Factor Alpha (TNF-a) and Lipopolysacccharide (LPS) Enhanced Natural Killer (NK) Cell Recruiting Chemokine Expression. Saeed Faramarzi, Mizanur Rahman, Nehir Ocak, Frederick Schatz, Charles J Lockwood. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Context: First trimester human decidua is comprised primarily of resident decidual cells (DCs), $CD56^{bright}$/CD16(-) decidual natural killer (dNK) cells, and macrophages. $CD56^{bright}$/CD16(-) decidual NK cells express high levels of latent cytototoxic molecules that can be rapidly activated to combat infectious microbes but also play a role in promoting trophoblast invasion. Host cell toll receptors (TLRs) mediate innate immunity. This study evaluated the effects of ligand-TLR-7 binding on TLR-4-ligand and macrophage-derived TNF-α stimulation of IP-10, a NK cell recruiting chemokine.
Objective: Assess effects of pre-treating DCs with Imiquimod (a TLR7 agonist) for 48 Hrs before incubating with either TNF-α, the TLR-4 agonist, LPS, or TNF-a + LPS on expression of IP-10.
Methods. Confluent, leukocyte-free first trimester DCs were primed for 7 days with 10^{-8}M estradiol 17β and 10^{-7}M medroxyprogesterone acetate. Cultures were then switched to a serum-free defined medium (DM) with the steroids and either with and without Imiquimod (5mg /ml) for 48 hrs before incubating with either TNF-α (1 ng/ml), LPS (5 mg/ml), or TNF-a + LPS. After 24 hr, conditioned DM supernatants were assessed by ELISA for IP-10.
Results: Table.

IP-10 raw data (pg/ml) for n=2 different decidual preparations (mean ± S.D.)

Conditions	Without TLR7 agonist	With TLR agonist
Control	4.0 ± 0	4.0 ± 0
TNF-α	893.1 ± 272.4	508.1 ± 91.8
LPS	38.5 ± 43.1	19.0 ± 15.5
TNF-α + LPS	1663.2 ± 359.6	480.7 ± 100.4

Conclusions: Macrophage-derived TNF-α and gram negative bacteria derived LPS may protect pregnancy by augmenting decidual cell expressed chemokines that recruit peripheral $CD56^{bright}$/CD16(-) NK cells to the decidua. Inhibition of this augmentation by pretreatment with the TLR-7 agonist Imiquimod suggests that specific viral infections in the decidua can inhibit peripheral NK cell recruitment to reduce resistance to subsequent bacterial decidual infections and potentially, impede NK cell stimulation of endovascular trophoblast invasion.

S-099

Pro-Inflammatory Signaling Pathway Mediation of IP-10 Expression in First Trimester Decidual Cells. Saeed Faramarzi, Mizanur Rahman, Nehir Ocak, Frederick Schatz, Charles J Lockwood. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

αBackground: First trimester human decidua, is comprised of decidual cells (DCs) (~50%), $CD56^{bright}$CD 16(-) uterine natural killer (uNK) cells (~30%), and macrophages (~10%). The chemokine IP10 promotes NK cell migration by acting as cognate ligands for CXCR3. Interferon gamma (IFNγ) is a primary NK cell product and tumor necrosis factor alpha (TNF-α) is a primary macrophage product.
Objectives: Elucidate the signaling pathway(s) that mediate changes in IP-10 expression in response to IFNγ and TNF-α separately and added together in cultured human first trimester DCs.
Methods: First trimester DCs were primed with estradiol or estradiol + medroxyprogestrone acetate in serum-containing medium then switched to a serum-free defined medium (DM) with the steroids. Cultured DCs were pre-treated with NFκB inhibitor (Curcumin) or STAT1 inhibitor (MTA) for one hour then incubated with control or IFN-γ, TNF-α, or IFN-γ +TNF-α for 24 hours (n=4). Cell lysates were used for western blotting to assess expression of NFκB, phospho (p)-NFκB, STAT1 and p-STAT1. A specific ELISA measured IP10 levels in conditioned DM supernatants.
Results: Western blotting demonstrated IFNγ enhanced p-STAT1 but not p-NFκB whereas TNF-α enhanced p-NFκB but not p-STAT1. The combination of IFNγ + TNF-α enhanced expression of p-STAT1 and p-NFκB.

IP-10 ELISA results in first trimester DCs

Conditions	No Inhibitor	NFκB Inhibitor (Curcumin)
Control	5.8 ± 1.8	4.0 ± 0
TNF-α	753 ± 224 *	51.4 ± 47.4 **
IFNγ	4,818 ± 3,460 *	187 ± 107 **
TNF-α + IFNγ	75,670 ± 28,157 *	6,017 ± 4,088 **

Data presented as mean ±SEM (pg/ml), n=4. * Compared to Control; ** compared to No Inhibitor; $p<0.05$.

Conclusions: At the implantation site, DC-mediated cross talk between IFNγ and TNF- synergistically enhances IP-10 expression. IFNγ enhanced IP-10 expression is mediated by phosphorylated NFκB and TNF-α enhanced IP-10 expression is mediated by phosphorylated Stat-1. Co-incubation of DCs with IFN-γ plus TNF-α and an NFκB inhibitor blocked the synergistic up-regulation of IP-10 expression. This DC -mediated crosstalk may recruit additional $CD56^{bright}$CD 16(-) NK cells to the decidua, where they promote several pregnancy protecting functions.

S-100

Preterm Birth: A Modulatory Role for HMGB1? Benjamin Miller, Amena Shelleh, John A Kirby, Stephen C Robson, Alison J Tyson-Capper. *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Context: Intrauterine Infection and Inflammation have been implicated as predisposing factors for preterm labour and preterm birth; this mechanism involves bacterial endotoxin binding to Toll-like receptors (TLRs) present on the surface of host cells triggering activation of a cascade of inflammatory events within the uterus. In the absence of infection, uterine and fetal inflammation still leads to preterm birth and neonatal injury. Endogenous ligands, including high mobility group box 1 protein (HMGB1) also bind to and modulate TLR activity.
Hypothesis: We hypothesise that HMGB1 plays a part in modulating inflammatory responsiveness linked with many cases of preterm labour.
Aims: To investigate the influence of HMGB1 on the expression and secretion of inflammatory proteins associated with TLR signalling in human myometrial smooth muscle cells.
Results: We first investigated the cellular distribution of HMGB1 within the human myometrium from non pregnant, preterm and term non-labouring and labouring women. Subcellular fractionation and immunoblotting showed that HMGB1 was most abundant in the nucleus in all samples, but lower levels were present in cytoplasmic and membrane fractions in myometrial samples prior to the onset of labour; compartment specific antibodies were included to assess purity of cell fractions. Primary myometrial cell cultures were next treated with recombinant HMGB1 protein (10ng-100ng/ml) or TLR4-specific LPS (10ng-1ug/ml) for 24 hours to assess their impact on expression and secretion of regulators and inflammatory proteins involved with TLR signalling. Immunoblotting data shows that HMGB1-treated uterine smooth muscle cells express much higher levels of the TLR regulatory protein interleukin-1 receptor associated kinase-M (IRAK-M) than untreated cells. Data from Proteome Profiler Arrays indicates that HMGB1-treated myometrial cells secrete a different profile of pro-inflammatory and anti-inflammatory cytokines and chemokines compared to endotoxin treated cells.
Conclusions: This work indicates that an endogenous ligand of TLR signalling may have the potential to modulate inflammatory responsiveness within the myometrium in the absence of infection.

S-101

Immune Cell-Influx into the Fetal Lung after Prolonged Exposure to *Ureaplasma parvum* Intra-Amniotic Infection (IAI). Jessica L Walker,[1] Amanda J Hamilton,[2] Victoria HJ Roberts,[1] Cindy E McEvoy,[2] Robert L Schelonka,[2] Peta L Grigsby.[1,3] *[1]Reproductive and Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR, USA; [2]Pediatrics, Oregon Health and Science University, Portland, OR, USA; [3]OB/GYN, Oregon Health and Science University, Portland, OR, USA.*

Objective: *Ureaplasma parvum* (*U.parvum)* contributes to preterm delivery and is associated with neonatal morbidities including bronchopulmonary dysplasia (BPD). The etiopathogenesis of BPD is an impairment of alveolar development which manifests as a chronic lung disease. We have previously shown that *U.parvum* IAI can cause fetal lung injury characterized by peribronchiolar lymphocytic aggregates and leukocytic infiltrates. Our objective was to characterize specific immune cells present in the fetal lung during *U.parvum* IAI.

Methods: Long-term catheterized rhesus monkeys received intra-amniotic inoculation of *U.parvum* (serovar 1; 10^7-10^8 CFU/mL) at 123-130 days gestation (dGA; term=168d; n=4). Gestational age-matched control animals did not receive *U.parvum* intra-amniotic inoculation (n=4). Lung tissues were obtained immediately following preterm cesarean section delivery, after prolonged *in utero* infection (14-21d). Fetal lung lobes were fixed with zinc formalin at 20cm H_2O constant pressure for 72h. Lung specimens were embedded in 4% agar, each lobe cut into randomized sections, paraffin embedded and sectioned at 5µm. Leukocytic infiltration of alveolar spaces, lymphocytic aggregates and septal wall thickness were qualitatively assessed. Immunohistochemical analysis included cell-specific antibodies for macrophages (CD68), B-cells (CD20cy) and T-cells (CD3).

Results: Fetal lungs exposed to prolonged *U.parvum* IAI demonstrated abundant macrophages, accompanied by lymphocytic infiltration of alveolar septal wall spaces. B- and T-cells were localized in the peribronchiolar aggregates and interstitial compartments. Macrophages were predominately localized in the alveolar spaces. Histologic appearance of control lung tissues showed thin septal walls and an absence of leukocytic debris in the airway spaces and an absence of lymphocytic aggregates.

Conclusions: We show that *U.parvum* IAI alters fetal lung development. The extent of the pro-inflammatory response detected in the preterm lung provides evidence that features of the BPD phenotype can be induced by *U.parvum* IAI with cellular changes beginning prior to delivery.

Support: R00HD055053, RR00163

S-102

Effect of *Lactobacillus rhamnosus* GR-1 on Cytokines and Chemokines in Maternal Plasma and Amniotic Fluid of Pregnant CD-1 Mice. SW Yang,[1,2,3] W Li,[1,2,3] JRG Challis,[1,2,3] SO Kim,[3] G Reid,[3] AD Bocking.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hospital; [2]Depts. Obstetrics and Gynecology and Physiology, University of Toronto; [3]Dept. Microbiology and Immunology, University of Western Ontario.*

Introduction: Preterm labor (PTL) occurs in 13% of all pregnancies in humans and accounts for 80% of neonatal mortality and morbidity. Probiotic *Lactobacillus* with immune-regulatory properties has the potential to confer health benefits by attenuating aberrations in the vaginal microbiota. We have shown GR-1 supernatant to increase the anti-inflammatory cytokine interleukin (IL)-10 production in human placental trophoblast cells while decreasing the production of pro-inflammatory cytokine tumor necrosis factor (TNF)-α. The effect of GR-1 on cytokine and chemokine secretion in pregnant mice, a well-described model for PTL, is unknown. **Objective**: Determine the effect of GR-1 on cytokine and chemokine secretion profiles in pregnant CD-1 mice. **Methods**: In two groups of pregnant mice, a daily dose of GR-1 at 10^8, 10^9 or 10^{10} cfu or saline was orally administered for 7 days from gestational day (gd) 9-15. The first group of mice (n=20) was monitored until term (gd 20). The second group of mice (n=31) was sacrificed on gd 16. Twenty one cytokines and chemokines in the maternal plasma (MP) and amniotic fluid (AF) were measured using Bioplex. Serum progesterone concentrations were determined using EIA. **Results**: Oral administration of GR-1 did not alter the gestational length and the MP progesterone level. However, in the MP, GR-1 (10^9 cfu) caused a significant increase in IL-6, IL-17, TNFα, and IL-10 concentrations from baseline levels of 200, 27, 210 and 150 pg/mL to 500, 300, 790 and 420 pg/mL respectively ($p<0.05$). No effect was seen at 10^9 cfu in the AF. A similar significant increase in IL-6, IL-17 and TNFα was detected in the AF but at a higher dose (10^{10} cfu) except for IL-10. GR-1 (10^{10} cfu) caused a significant increase in the chemokines KC and MIP-1β in the AF from 1050 and 75 pg/mL to 8100 and 430 pg/mL respectively ($p<0.05$), but not in the MP at any dose. Conclusion: GR-1 administered orally to pregnant CD-1 mice alters the innate immunity, leading to differential cytokine and chemokine profiles in the MP and AF. Compared to MP, a higher dose of GR-1 is needed to induce changes in cytokines and chemokines in the AF. These findings support in vitro studies and further suggest that lactobacilli might counter inflammation-associated PTL.

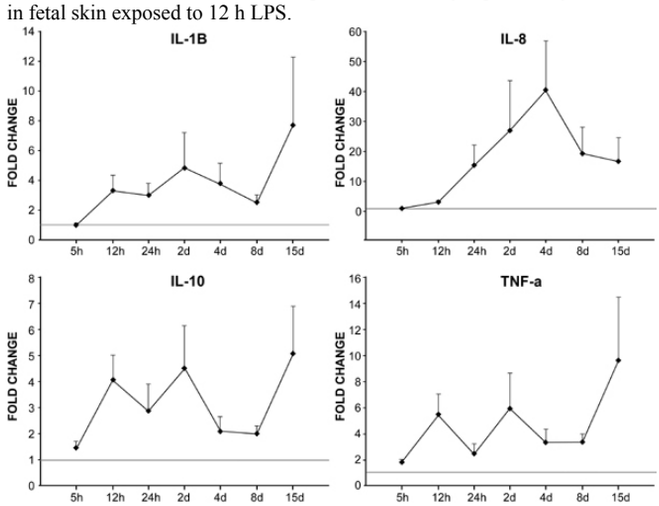
S-103

***In Utero* Exposure to *E. coli* LPS Induces Prolonged Cytokine Expression in the Fetal Ovine Skin.** Li Zhang,[1,4] Masatoshi Saito,[1] Alan Jobe,[1,2] Suhas Kallapur,[1,2] John Newnham,[1] Thomas Cox,[1] Shaofu Li,[1] Boris Kramer,[3] Huixia Yang,[4] Matthew Kemp.[1] *[1]School of Women's and Infants' Health, Univ of Western Australia, Australia; [2]Division of Pulmonary Biology/Neonatology, Cincinnati Children's Hospital, USA; [3]Dept Pediatrics, Maastricht Univ Medical Center, Netherlands; [4]Dept OB/GYN, Peking Univ First Hospital, China.*

Background: Preterm birth (PTB) is associated with *in utero* infection and inflammation. The origins and progression of the inflammation associated with PTB are still unclear. In fetal sheep, intraamniotic (IA) injection of LPS induces the expression of pro-inflammatory cytokines in the skin and the lung 2d after exposure with a decrease to control levels by 7d in the lung. The kinetics of pro-inflammatory cytokine expression in the skin are unstudied. We used an ovine model to ask if the fetal skin was capable of generating an extended response to inflammation stimuli.

Methods: Date mated merino ewes were divided into two groups: IA 2 ml saline (control,n=5); and IA 10 mg LPS from O55:B5 *E.coli* in 2 ml saline for either 5 h, 12 h, 24 h, 2 d, 4 d, 8 d, or 15 d (n=7). Fetuses surgically delivered at 124 d gestational age (± 2 d; term=150 d). Fetal skin from the inner left thigh was collected for quantitative PCR analysis of cytokine expression.

Results: Relative to control, LPS exposure induced significant increases in (fold change) IL-1β (2 d, 4 d, 15 d), IL-8 (24 h, 2 d, 4 d, 8 d, 15 d), IL-10 (12 h, 2 d, 15 d) and TNFα (12 h, 2 d, 8 d, 15 d) mRNA expression [Figure 1]. IL-6 , MCP-1 and TLR-4 mRNA expression was only significantly increased in fetal skin exposed to 12 h LPS.



Conclusions: The fetal skin is highly vascularised, comprises a significant proportion of the fetal surface area and is exposed in its entirety to the amniotic environment. In sharp contrast to the lung, skin expression of pro-inflammatory cytokines continues unabated 15 d after exposure to IA LPS. These novel data demonstrate that the fetal skin is capable of generating a prolonged response to bacterial agonist and suggest the fetal skin plays an important role in generating the *in utero* inflammation causally associated with PTB.

S-104

A Potential Correlation between Antenatal Maternal Stressors and Irritable Bowel Syndrome/Functional Abdominal Pain in Offspring. Melanie E Arndt,[1] Diana A Racusin,[1] Erica M Weidler,[2] Haleh Sangi-Haghpeykar,[1] Robert J Shulman,[2] Kjersti M Aagaard.[1] *[1]Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Pediatrics-Nutrition, Baylor College of Medicine, Houston, TX, USA.*

OBJECTIVE: It is often suggested that maternal stress during pregnancy may be detrimental to her offspring. However, objective data employing measurable disease endpoints or traits in children has been scarce. In adults, irritable bowel syndrome (IBS) and functional abdominal pain (FAP) have been linked to psychosocial stressors. We hypothesized that IBS and FAP in children may have

their origin in fetal life. Our aim in this pilot study was to provide an initial investigation into the possible connection between quantifiable and objective antenatal events and IBS/FAP occurring in young life.
STUDY DESIGN: We performed a casecontrol pilot study from children in a regional pediatric GI clinic, and with a prior and extensively well-characterized diagnosis of IBS/FAP (pediatric Rome III criteria). In total, 36 children were enrolled and were of comprised 9 FAP/IBS cases and 27 controls from 7 sibling clusters and two sets of twins. Data was abstracted from released records across the subjects lifespan, including antental care records, L&D and newborn data, and extensive previously validated parental and child questionairres. For determination of significance, and nominal p value <0.05 was accepted (students t-test for continuous data and Fischer exact for group data; SAS).
RESULTS: Cases and controls did not differ by virtue of maternal age (29.56 vs 29.63, p=0.97), gestational age at delivery (38.08 vs 38.55 weeks, p=0.65), nor race/ethnicity, marital status, delivery mode, pregnancy comorbidities, newborn course, breastfeeding, nor newborn feeding problems (p>0.23). IBS/FAP was more common with a maternal psychiatric diagnosis (OR 4.0, 95%CI 0.61,27.01) and reported or recalled major psychosocial stressors during the index case pregnancy (OR 3.6, 95%CI 0.75,18.47). Maternal stressors ranged from unemployment and financial to incarceration.
CONCLUSION: In this initial case-control study inclusive of sibling and twin pair sets, we observed that one potential contributor to pediatric IBS/FAP may include subjective and objective measures of maternal stress. Future longitudinal cohort studies are likely warranted.

S-105

Elective C-Section or Trial of Labor after Cesarean Section: Factors Affecting Maternal Choice. Sarah N Bernstein, Shira Grazi-Matalon, Barak Rosenn. *Obstetrics and Gynecology, St. Lukes-Roosevelt Hospital Center, New York, NY, USA.*
Objective: We sought to determine the factors that affect maternal choice of mode of delivery in women who are candidates for a trial of labor after cesarean (TOLAC).
Study design: This was a prospective, observational study of women who presented for delivery between November 2010 and July 2011 with a history of one prior low-transverse c-section (CS) and no contraindications for TOLAC. Consenting women filled out a questionnaire upon presentation for their scheduled elective repeat CS or upon admission for their TOLAC. Chi-Square and t-test were used, as appropriate, with Bonferroni correction for multiple comparisons.
Results: The study included a total of 155 women, 87 for TOLAC and 68 for CS. There were no differences between groups with respect to age, education, ethnicity, and provider type, as well as the indication for the prior CS and planned family size. 49% of women in the TOLAC group had either a previous vaginal delivery or successful TOLAC compared to only 16% in the CS group. Additionally, women in the TOLAC group were more likely to be dissatisfied with their previous CS, state that their previous recovery was harder than expected, expected their pain to be better controlled with TOLAC, and state that their provider preferred a TOLAC. When asked to rank the importance of various factors in their decision making process, there were no differences between groups regarding their consideration of maternal and neonatal safety, cosmetic outcome, fear of vaginal damage, plans to have a large family, importance of partner's or doctor's opinion, or desire to experience a vaginal delivery.
Conclusion: Maternal experience in a prior CS and the perceived preference of the care provider are the main factors that determine a woman's choice with respect to TOLAC. Better education of providers regarding the benefits of TOLAC may contribute to a decrease in the high rate of elective repeat CS.

Factor	TOLAC (n=87)	ERCS (n=68)	P Value
Prior Successful VBAC	N=19	N=4	.0056
Prior Successful Vaginal Delivery	N=24	N=7	.0076
Satisfied with Prior Cesarean	N=27	N=32	.041
Dissatisfied with Prior Cesarean	N=17	N=1	.0004
Pain Poorly Controlled after previous cesarean	N=14	N=1	.0022
Pain will be better controlled in repeat cesarean	N=26	N=51	<.00001
Pain will be better controlled in TOLAC	N=56	N=8	<.00001
Recovery Harder than expected	N=51	N=23	.0021
Doctor Preferred TOLAC	N=36	N=10	.0003
Doctor Preferred Repeat Cesarean	N=3	N=19	.000011

S-106

Obstetrical Management of Women Referred to Labor and Delivery Despite Normal Antepartum Testing. Justin S Brandt,[1] Pooja Gala,[2] Stephen T Chasen.[3] *[1]Obstetrics and Gynecology, New York Presbyterian Hospital - Cornell; [2]Obstetrics and Gynecology, Weill Cornell Medical Center; [3]Obstetrics and Gynecology, New York Presbyterian Hospital - Cornell.*
Objective:
Some patients undergoing antepartum testing will have abnormalities on fetal monitoring despite having reactive non-stress tests (NST) or normal biophysical profiles (BPP). In our institution, these patients may be referred to labor and delivery (L&D) for further evaluation. Our objective was to determine how these patients are managed on L&D.
Study Design:
We reviewed the records of women who underwent NST or BPP at our institution from 2009-2011. We identified those patients who were referred to L&D for further evaluation of periodic or episodic decelerations in fetal heart rate tracings despite reactive NST or normal BPP of 8/10 or 10/10. We excluded women referred to labor and delivery for hypertension, oligohydramnios, contractions, leakage of fluid, and status post amniocentesis or external cephalic version.
Results:
During the study period, 12,427 NST, with or without BPP, were performed at our institution. There were 128 women with reactive NST or normal BPP who were referred to L&D (1 in 97). The mean gestational age was 38.4 weeks. 66/128 women (51.6%) were monitored in triage and then discharged home with reactive NST and normal amniotic fluid volumes. 41/128 women (32%) underwent labor induction or cesarean delivery as a result of antepartum testing. 21/128 women (16.4%) were admitted to the labor floor for prolonged monitoring. Of 62 women who were were delivered or admitted for prolonged monitoring, 35 (56.5%) had cesarean deliveries.
Conclusions:
The majority of patients with reactive NST or normal BPP who were referred to L&D for further evaluation were discharged home. A significant proportion of patients were considered to have non-reassuring fetal status necessitating delivery. Given the low false negative rates of antepartum testing, these findings may reflect a low threshold to deliver patients at term with fetal heart rate decelerations despite reassuring fetal testing. As these patients had a high rate of cesarean delivery, further study is necessary to evaluate the benefits of this approach.

S-107

Severe Preeclampsia at 32 to 34 Weeks, Management, and Maternal and Neonatal Outcomes. Margaret Carter, Rebecca Uhlmann, Giancarlo Mari. *Department of Ob/Gyn, Division of Maternal Fetal Medicine, University of Tennessee Health Science Center, Memphis, TN, USA.*
In the setting of severe preeclampsia (SPreE), most would agree with expectant management at <32 weeks and immediate delivery at >34 weeks. The decision to deliver verses expectant management between 32 to 34 weeks is controversial, so we sought to evaluate the maternal and neonatal risks associated with pregnancy prolongation beyond 32 weeks but <34 weeks in the setting of SPreE . To conduct this study, we gathered data from our obstetrical database on deliveries in our hospital from 1990 to 2005. From the patients who delivered between 32 0/7 and 33 6/7 weeks (n=2680), we identified those who were admitted with a diagnosis of SPreE, eclampsia, HELLP syndrome, or chronic hypertension and were delivered during the same admission (n=96). We determined the time from admission to delivery and divided the patients into three groups--immediate management (delivery within 1 day; n=49), active management (>1 day but <3 days; n=28), and expectant management (>3 days; n=19). Delivery indications--eclampsia, chorioamnionitis, abruption, labor, oligohydramnios, intrauterine growth restriction (IUGR), non-reassuring fetal heart rate testing (NRFHT), intrauterine fetal demise (IUFD), HELLP syndrome, and superimposed preeclampsia (SIP)--were used to assess maternal and neonatal outcomes. To further assess neonatal outcome, we looked at intubation at delivery, nursery disposition, and 5 minute Apgar score<7. The immediate group was compared to the active and expectant groups by outcome using chi-square analyses. Statistical significance was set at p≤0.05. In the active group, there was a significantly higher rate of indicated deliveries for oligohydramnios (p=0.01) and NRFHRT (p=0.04) and an increased rate of neonatal intubation at delivery (p=0.01). In the expectant group, there was a significantly increased rate of labor progression (p=0.02) and worsening of SIP (p=0.02). Based on our limited study, immediate management verses active or expectant management in the setting of SPreE does not necessarily worsen maternal or neonatal outcome. In our sample, more women did not

develop eclampsia, abruption, chorioamnionitis, or HELLP syndrome; and, more fetuses did not develop IUGR or become an IUFD. Also, more neonates did not go to the level 2 or 3 nursery or have a 5 minute Apgar score<7 in the immediate group. Therefore, no particular management can be supported fully without additional studies.

S-108

Evaluating Antenatal Corticosteroid Administration as a Measure of Obstetric Quality. Suchitra Chandrasekaran, Jamie A Bastek, Meghan McShea, Markley Foreman, Michal A Elovitz, Sindhu K Srinivas. *Perelman School of Medicine, Maternal and Child Health Research Program, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: Antenatal corticosteroids (ACS) decrease the morbidity and mortality of preterm infants. The joint commission is now requiring all hospitals to report ACS administration as a core measure of perinatal care. We assessed the rate of ACS administration in patients who presented with signs/symptoms of preterm labor (PTL), and subsequently had a preterm birth (PTB) at <34 weeks of gestation as a measure of quality of obstetric care.

STUDY DESIGN: We performed a retrospective study within a prospective cohort study of women with singleton pregnancies, who presented at 22-33 6/7 wks with symptoms of preterm labor (4/09 – 6/11) at a single urban institution. Maternal history and antenatal data were recorded including date and time of ACS administration, and delivery by chart abstraction. Descriptive statistics were performed. Categorical variables were compared using Chi-square analyses or Fisher's Exact tests as appropriate.

RESULTS: 595 women were included. 14.5% (N=86) delivered < 34 weeks and 95.3% of them received ACS. There was no difference in maternal race (p=0.53) or age (p=0.39) between groups. There were also no differences in median gestational age (p=0.45) or delivery mode (p=0.68). Of the 82 women who received ACS, 92.6% received them on the day of admission and all received two doses. The median time (MT) from admission to ACS was 136 minutes (IQR: 79-240 minutes). The MT from ACS to delivery was 3 days (IQR: 1-9 days). 3 of the 4 women who did not receive ACS delivered between 10 and 100 minutes of admission.

CONCLUSION: The majority of patients in this cohort who had a PTB received ACS. However, there is still room for improvement both in time from admission to administration as well as in administration to those who have a short interval from admission to delivery. Further research is needed to evaluate the rate of steroid administration in all preterm deliveries <34 weeks and to develop measurement tools and systems based approaches to maximize the administration of this beneficial therapy.

MOD#21FY08-539 (Elovitz)

S-109

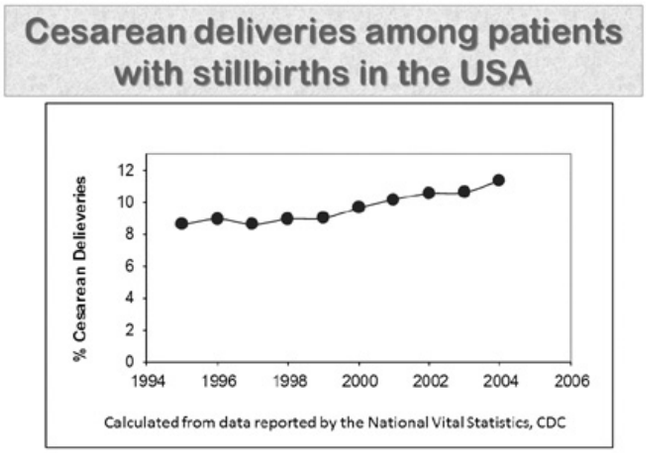
Temporal Changes in Cesarean Delivery Rates among Patients with Stillbirths: A Population Based Study in the United States. Tally Faro,[1] Joaquin Santolaya-Forgas,[1] Valeria Di Stefano,[1] Cande V Ananth.[2] *[1]Obstetrics and Gynecology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA; [2]Obstetrics and Gynecology, Columbia University, New York, NY, USA.*

Objectives: To determine the changes in rates of cesarean deliveries among women with stillbirths.

Study Design: We used the natality and fetal mortality data files, assembled by the National Center for Health Statistics (NCHS) of the United States Centers for Disease Control and Prevention. We reviewed data from the years 1995 to 2004 and selected the entries to calculate the rate of cesarean deliveries after 20 weeks gestation among patients with stillbirths.

Results: During the study period there were 35 million births that met the entry criteria into the study of which 205,672 were stillbirths. The rate of cesarean section among these pregnancies increased from 8.6% in 1995 to 11.3% in 2004 (~40%). Figure 1 depicts the trend of changes in cesarean delivery rate over the study period.

Conclusion: Cesarean deliveries in pregnancies with stillbirths increased over a ten year period. This novel epidemiologic finding can be used for the formulation of hypotheses to understand why this happend.



S-110

Association between Asthma during Pregnancy and Adverse Perinatal Outcome Based on Maternal Prepregnancy BMI and Race/Ethnicity Disparity. Michael J Fassett,[1] Darios Getahun.[2] *[1]Maternal-Fetal Medicine, Kaiser Permanente West Los Angeles Medical Center, Los Angeles, CA, USA; [2]Research and Evaluation, Kaiser Permanente Southern California, Pasadena, CA, USA.*

Objective: To examine if obesity modifies the risk of adverse perinatal outcome in a pregnancy complicated by asthma and whether the risk varies by maternal pre-pregnancy BMI and race/ethnicity.

Methods: We studied all singleton pregnancies delivered at ≥20 weeks of gestation (n=182,848) in Kaiser Permanente Southern California (KPSC) hospitals (1993-2009). Prepregnancy BMI (kg/m^2) was categorized as underweight (<18), normal weight (18.5–24.9), overweight (25-30), and obese (BMI ≥ 30). Odds ratios (OR) and 95% confidence intervals (CI) were used to estimate associations after adjusting for potential confounders.

Results: Compared with normal weight women, obese women were more likely to be older, from Hispanic or African-American ethnic/racial groups, to have ≤12 years of education, and smoked during pregnancy. Overweight and obesity were associated with 3.6-fold (95%CI 2.5-5.2) and 1.8-fold (95%CI 1.2, 2.8) increased risk of placental abruption and with 1.2-fold (95%CI 1.1, 1.5) and 1.3-fold (95%CI 1.2-1.5) increased risk of preeclampsia, and with 2.6-fold (95%CI 1.1-6.1) and 2.7-fold (95%CI 1.5-4.5) increased risk of extremely preterm birth, respectively. Among underweight women, asthma was associated with increased risk of IUGR (OR 5.9, 95% CI 3.1, 11.2) and spontaneous preterm birth (OR 2.9, 95% CI 1.6-5.2). We observed a significant heterogeneity in BMI effect in the relationship between asthma and adverse perinatal outcome by race/ethnicity. In overweight and obese women, asthma was associated with increased risk of abruption in Asian/Pacific Islanders (OR 4.9, 95% CI 2.5-9.8).African-Americans (OR 3.4, 95%CI 2.0-5.7), Hispanics (OR 2.5, 95% CI 1.7-3.7), but not Whites (OR 0.8, 95% CI 0.3-2.2); and preterm birth in African-Americans (OR 1.9, 95% CI 1.5-2.3), Whites (OR 1.4, 95% CI 1.2-1.7), but not Hispanics (OR 1.2, 95% CI 1.0-1.3) or Asian/Pacific Islanders (OR 0.9, 95% CI 0.6-1.5).

Conclusion: The results suggest that among pregnant women with asthma, there is considerable heterogeneity in risk for adverse perinatal outcome based on pre-pregnancy BMI categories.

S-111

Cord Gas Analyses in Relation to Neonatal Morbidity and Mortality: The EveREst Plot. Antoniya Georgieva, Christopher WG Redman. *Obstetrics and Gynaecology, University of Oxford.*

Background: The thresholds of cord arterial pH and Base Deficit (BD) signifying higher risk for neonatal morbidity/mortality (NMM) are uncertain (Malin GL. BMJ 2010;340). Whether pH or BD are better predictors of NMM is unclear (Wiberg N. AJOG 2006;195). The incidence of NMM is low and its association with cord gases highly nonlinear. Standard ROC curve analyses only show a weak association but do not provide any further insight.

Objective: To evaluate the relation of cord pH and BD to NMM.

Methods: 34,510 term singleton deliveries were studied (Fig 1). A new method to evaluate cord gases against outcomes is described - Event Rate Estimate

(EveREst) plots, Fig 2: cord gas values are sorted in descending (pH) or ascending (BD) order and grouped into exclusive quantiles, then plotted against % rates for NMM.

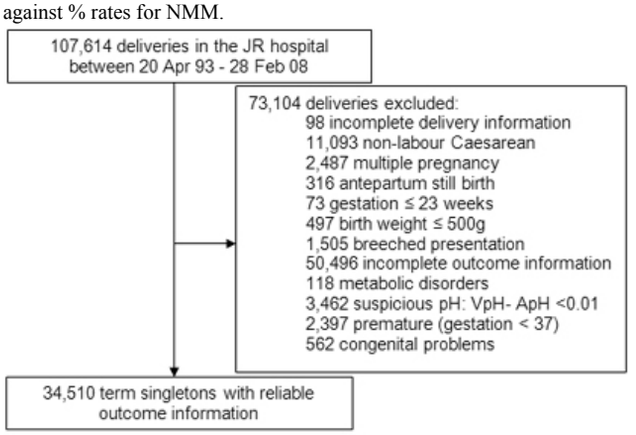
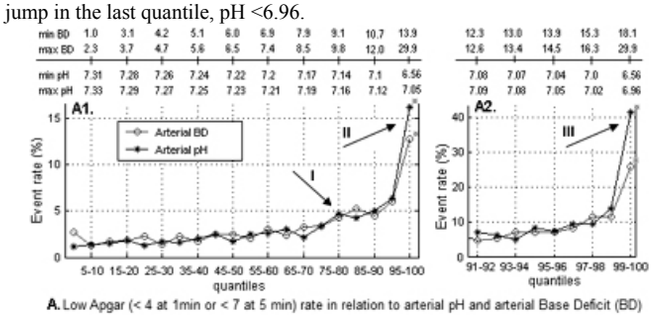


Figure 1. Patient selection.

JR hospital – John Radcliffe Hospital, Oxford; VpH – venous pH; ApH – arterial pH

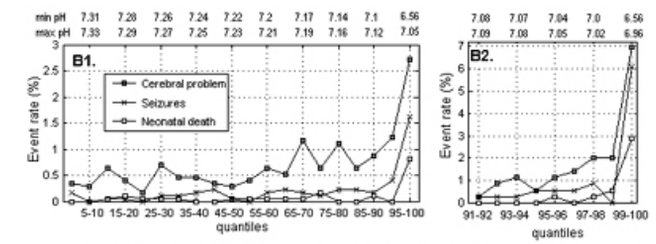
Results: In non-acidemic babies, rates of low Apgars were relatively steady but rose modestly at arrow I (Fig 2-A1) and sharply at arrow II, with increasing acidemia. Closer inspection showed that the incidence of low Apgar rose suddenly in the last quantile (Fig 2-A2, arrow III). pH and BD gave similar event rates until the last quantile, where pH was significantly better.

Only pH is shown in relation to other neonatal outcomes (Fig 2-B). The rates for cerebral problem (e.g. haemorrhage, ischemia, leukomalacia) rose from 0.5% in the non-acidemic cases, to 2% for pH between 6.96 and 7.02, and up to 7% in the last quantile. The death rates rose for pH <7.02, with a sudden jump in the last quantile, pH <6.96.



A. Low Apgar (< 4 at 1min or < 7 at 5 min) rate in relation to arterial pH and arterial Base Deficit (BD)

* p < 0.001, χ2 test for difference in proportions, compared are the event rates for pH and BD



B. Neonatal cerebral problem (ischemia, leukomalacia, irritability , depression or coma), seizure and death rates (ICD9 or ICD10 codes) in relation to arterial pH

Figure 2. Event Rate Estimate (EveREst) of the relation between blood gases and selected adverse outcomes: A1, B1 over the full range; A2, B2 expanded for the upper 10 quantiles

Conclusion: EveREst plots display clearly the diagnostic value of measures such as cord gases that detect rare events, providing a 'map' for the relevant risks. The relation of blood gases to NMM is consistent and gives continuous grading of the risk for neonatal compromise. Very low pH better reflects the risk of NMM than very high BD.

S-112

Association of Subclinical Hypothyroidism with Adverse Outcomes in Pregnancies Complicated by Diabetes Mellitus. Katherine Campbell, Unzila Ali, Lisa Zuckerwise, Christian M Pettker, Erika F Werner, Stephen F Thung, Christina S Han. *Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University, New Haven, CT, USA.*

Objective: Subclinical hypothyroidism is associated commonly with pregestational diabetes mellitus and has been linked to impaired fetal neurodevelopment. However, unlike overt thyroid disorders in pregnancy, the association of subclinical hypothyroidism to adverse pregnancy outcomes, such as preeclampsia and preterm delivery, remains uncertain. The objective of this study is to evaluate the utility of routine screening of diabetic pregnancies for thyroid disease in the prediction of adverse pregnancy outcomes.

Methods: We examined a retrospective cohort of pregestational diabetic women who delivered at Yale-New Haven Hospital from 2007-2010. Thyroid stimulating hormones (TSH) were obtained at the first prenatal visit. TSH > 2.5 IU/mL was used as a threshold for subclinical hypothyroidism. Common adverse outcomes, such as preeclampsia, preterm delivery before 34 weeks gestational age and birth weight greater than 4000 grams were evaluated. Logistic regression was used to adjust for confounders including pre-existing thyroid disease, maternal age, race, and hemoglobin A1c.

Results: Of 217 patients with pregestational diabetes, TSH values were available in 187 patients. TSH > 2.5 uIU/mL was found in 23 (12.2%) patients and TSH > 3.5 uIU/mL was found in 12 (6.4%) patients. After adjusting for confounders, TSH >2.5 uIU/mL was not associated with differences in the rates of preeclampsia (adjusted OR 1.96, p=0.12), preterm delivery before 34 weeks (adjusted OR 0.42, p=0.1) and birth weight greater than 4000 grams (adjusted OR 0.6, p=0.36). TSH > 3.0 and 3.5 uIU/mL were also not associated with the above outcomes.

Conclusion: TSH levels in pregestational diabetic women did not identify a group at risk for common adverse outcomes associated with diabetes in pregnancy.

S-113

Maternal Intensive Care Unit (ICU) Admission in a Contemporary OB Population. Amy C Hermesch, Judith Hibbard. *Obstetrics and Gynecology, University of IL, Chicago.*

Objective: With increasing maternal age and rising induction and cesarean (CD) rates in the US, we were interested to examine a contemporary OB cohort for maternal factors related to post partum (pp) ICU admission.

Methods: Data were obtained from the Consortium on Safe Labor, a multicenter electronic OB database. A total of 179,666 patients were included from study sites that provided information on maternal pp ICU admission. The primary interest group were those women admitted to the ICU in the immediate pp period and controls were women not admitted to an ICU. Baseline demographics were compared. Rates of ICU admission by pre-pregnancy comorbidities (diabetes, chronic hypertension, heart disease, gastrointestinal disorders, depression, seizure disorder, asthma, anemia and HIV/AIDs) were also compared. Mode of delivery was categorized by: spontaneous labor leading to either vaginal delivery (VD) or cesarean delivery (CD) or induction of labor (IOL) leading to either VD or CD. Elective CDs without labor were also studied. Statistical analyses were performed using t-tests and chi-squares. Multivariable logistic regressions were used to calculate adjusted ORs and 95% CIs.

Results: A total of 1,003 women were admitted to the ICU and 178,663 women were not. Maternal age, gravity, parity, BMI were not significantly different. After multivariable logistic regression analysis controlled for maternal demographic factors; we found several pre-pregnancy co-morbidities associated with ICU admission, including diabetes, chronic hypertension, and history of heart disease. Among the patients admitted to the ICU, IOL leading to vaginal delivery conferred a 70% increased risk for ICU admission.

Mode of delivery in women admitted to the ICU postpartum versus not admitted to the ICU

	ICU admission	Non-ICU admission	Odds Ratio	Significance
	% (n)	% (n)	OR (95%CI)	p
Spontaneous labor leading to VD	33.1 (332)	40.4 (72,195)	0.7 (0.6 – 0.8)	< 0.001
IOL leading to VD	41.7 (418)	29.5 (52,774)	1.7 (1.5-1.9)	< 0.001
Spontaneous labor leading to CD	3.7 (37)	5.6 (10,031)	0.6 (0.5-0.9)	< 0.05
IOL leading to CD	4.3 (43)	7.7 (13,717)	0.5 (0.4-0.7)	< 0.001
Elective/scheduled CD	17.2 (173)	16.8 (29,946)	1.0 (0.9-1.2)	= 0.680

Conclusions: ICU admission was most likely among women who had pre-pregnancy diabetes, chronic hypertension or history of heart disease and among women with IOL that led to VD. We suggest that IOL should be limited to those with compelling indications only.

S-114

Stillbirth Gestational Age as a Predictor of Recurrence Risk. Cara C Heuser,[1] Molly McFadden,[2] Michael Varner,[3] Robert M Silver.[3] [1]*Maternal Fetal Medicine, Intermountain Healthcare and University of Utah, Murray, UT, USA;* [2]*Internal Medicine, University of Utah, Salt Lake City, UT, USA;* [3]*Maternal Fetal Medicine, University of Utah.*

Objective: Women with a previous stillbirth (SB) are known to be at increased risk of subsequent stillbirth. Recurrence risk may be different for stillbirths at different gestational ages, but this has not been examined. Our purpose was to evaluate the risk of subsequent SB according to the gestational age of initial SB.

Methods: Retrospective cohort study using the Utah Population Database (UPDB), a collection of vital statistics with the capability of linking birth and death records. We included all women delivering a singleton stillbirth (≥ 20 weeks gestation) in Utah from 1989-2005 with at least one subsequent pregnancy ≥ 20 weeks gestation. We stratified the initial SB by 4-week increments of gestational age and assessed the relative risk of subsequen SB. We included in the analysis up to 3 births after the initial SBs because at 4 births or greater the scarcity of data precluded analysis. We controlled for parity and maternal age.

Results: 2896 mothers and 5095 subsequent births qualified. Of the subsequent births, 100(1.96%) were SBs. Due to missing data on maternal age, only 5088 births and 94 SBs were used in the analysis. Table 1 shows relative risk of subsequent SB based on the gestational age of initial SB. With the exception of the group of women in whom the initial SB occurred at greater than 40 weeks, women with an early initial SB suffered earlier subsequent SBs as compared to women with later initial SBs (effect of gestational age p=0.002).

Gestational age at initial SB	Subsequent SB n(%)	Relative Risk of Recurrence (95%CI)	Mean gestational age at subsequent SB (p25-75)
20 to <24w	42(2.7)	3.4 (1.7-7.0)	23.1 (20-24)
24 to <28w	14(1.9)	2.5 (1.1-6.0)	25.4 (21-28)
28 to <32w	12(2.3)	3.2 (1.4-7.4)	26.1 (22.5-29)
32 to <36w	13(1.8)	2.2 (0.9-5.5)	26.7 (22-32)
36 to <40w	10(0.8)	1.0 (referent)	32.0 (27-27)
40+w	9(2.8)	3.5 (1.3-9.4)	27.9 (21-37)

Conclusion: Gestational age at initial SB predicts risk of recurrent SB. This effect is most pronounced in women with very preterm or with post-term pregnancies with over three times the recurrence risk as SB at term. These data have important implications for counseling patients and will contribute to the ultimate goal of understanding the cause of and working toward preventing this devastating pregnancy complication.

S-115

Current Decision Rules in Fetal Surveillance Are Suboptimal. JJ Kaandorp,[1] AJ Mesker,[1] S Loix,[2] F de Boer,[3] E Kentson,[4] M Kleppe,[5] JH Baalman,[1] E Schuit,[6] MJ Benders,[1] GH Visser,[1] JB Derks,[1] BW Mol.[7] [1]*Perinatology, University Medical Center, Utrecht, Netherlands;* [2]*Obstetrics, Maxima Medical Center, Veldhoven, Netherlands;* [3]*Obstetrics, University Medical Center, Groningen, Netherlands;* [4]*Obstetrics, Groene Hart Hospital, Gouda, Netherlands;* [5]*Obstetrics, Maastricht University Medical Center, Netherlands;* [6]*Julius Center for Health Sciences, University Medical Center, Utrecht, Netherlands;* [7]*Obstetrics, Academic Medical Center, Amsterdam, Netherlands.*

Preventing intrapartum fetal asphyxia remains one of the biggest challenges in perinatal medicine. Of all Caesarean sections and instrumented vaginal deliveries performed for the indication fetal distress, only 2-5% of all children will indeed have metabolic acidosis. We aimed to determine which clinical characteristics are associated with a higher risk of a low umbilical pH in women that undergo an instrumental delivery for fetal distress.

Methods We studied consecutive term patients who underwent a Caesarean section or assisted vaginal delivery for the indication fetal distress in 6 Dutch hospitals from October 2010 to August 2011. We assessed the association between an umbilical artery pH < 7.10 and the characteristics maternal age (yrs), parity, gestational age (wks), previous Caesarean section, intra uterine growth restriction (IUGR), maternal fever, diabetes, highest diastolic blood pressure and induced onset of labor using univariable and multivariable logistic regression.

Results Of 707 patients studied, 114 (16%) had a child with an umbilical artery pH < 7.10. In the univariable analyses a low umbilical pH was associated with maternal age (OR 1.05 [1.03-1.06]), nulliparity (OR 0.58 [0.51-0.66]), previous Caesarean section (OR 1.27 [1.04-1.55]), IUGR (OR 0.46 [0.34-0.63]), maternal fever (OR 0.76 [0.59-0.97]) and diabetes (OR 2.02 [1.64-2.48]). Multivariable analysis yielded that maternal age (OR 1.03 [0.98-1.08]) and diabetes (OR 1.86 [0.92-3.75]) were predictive for an umbilical pH < 7.10, while nulliparity (OR 0.64 [0.41-1.01]) and IUGR (OR 0.50 [0.18-1.41]) were associated with a decreased risk.

Conclusion In term women who undergo intervention for suspected intrapartum fetal distress, the risk profile is not stable. Since it would be ideal that every woman would have a similar threshold for intervention for fetal distress, our data show that current decision rules in fetal surveillance are suboptimal.

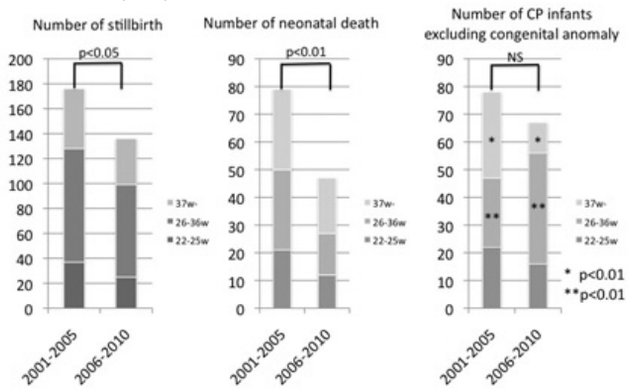
S-116

Trends in Cerebral Palsy: A Regional Population-Based Data in Southern Japan. Yuki Kodama, Hiroshi Sameshima, Koutarou Doi, Tsuyomu Ikenoue. *Obstetrics and Gynecology and Perinatal Center, University of Miyazaki, Kihara, Kiyotake-cho, Miyazaki, Japan.*

OBJECTIVE: Cerebral palsy (CP) prevalence has not decreased in developed countries over 30 years. Improved survival rates for extremely preterm infants who are at particular risk of neurological damages may be attributed. The aim of this study was to assess overall gestational age-specific trends in the rate of CP in our population, where the perinatal mortality rate is 3.0 per 1,000.

STUDY DESIGN: We performed an unselected population-based study in Miyazaki (10,000 deliveries /year), where 102,999 cases were born between 2001 and 2010. We hold peer-review audit conferences and examined all of the 312 stillbirths (at > 22 weeks), 126 neonatal deaths, and 214 infants having a risk of CP. Infants were classified into 3 groups according to gestational age; term (>37 weeks), moderately preterm (26-36 weeks), extremely preterm (22-25 weeks). Prevalence of Infant survival and neurological damage ware compared in two 5-year periods, 2001-2005 and 2006-2010.

RESULTS: The number of stillbirth and neonatal death decreased significantly during the 2 periods (figure). The overall prevalence of CP was 2.1/1000. The number of CP also decreased from 126 to 88. After excluding congenital anomalies, the corrected CP prevalence was 78/51,889 (1.5 per 1,000) and 67/51,110 (1.3 per 1,000), which did not reach statistically significant (figure). Among them, extremely preterm infants did not differ, moderately preterm infant increased, and term infant decreased (p<0.01, figure). In term infants, asphyxia decreased from 18 to 7. In moderately preterm infants, periventricular leukomalacia (PVL) increased from 12 to 21.



CONCLUSION: According to our unselected population-based data, perinatal death and CP decreased during the 2 study-periods. Term CP infants were decreased mainly due to the reduction of asphyxia. PVL may attribute to the increase of CP in preterm infants.

S-117

Attempt To Quantify to Uterine Involution Using Acoustic Radiation Force Impulse (ARFI) before and after Placental Delivery. Shintaro Makino, Toshitaka Tanaka, Takashi Yorifuji, Taro Koshiishi, Motoi Sugimura, Satoru Takeda. *Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo, Japan.*

Objectives: Acoustic radiation force impulse (ARFI), a technique to determine the hardness of soft tissue by causing micro-displacement with acoustic radiation pressure and ultrasonically quantifying it, has recently been applied to ultrasonographic systems, and this has become clinically available. Thus, in the present study, the hardness of the uterine corpus and cervix was quantified using ARFI before and immediately, 1, and 2 hours after placental delivery in order to examine the change in hardness over time.

Methods: The study subjects consisted of 11 patients who delivered at our hospital. An *ACUSON S2000* (Mochida Siemens Medical Systems Co., Ltd., Tokyo, Japan) equipped with ARFI was used as an ultrasonographic system.

The hardness of the uterine corpus and cervix muscularis was measured at a depth of approximately 15 mm from the uterine surface using a transabdominal probe before and immediately, 1, and 2 hours after placental delivery.

Results: The hardness of the uterine corpus before and immediately, 1, and 2 hours after placental delivery was 1.81 ± 0.60, 3.04 ± 0.76, 3.12 ± 0.95, and 2.72 ± 0.81 m/s, and the hardness of the uterine cervix was 1.35 ± 0.45, 1.87 ± 0.57, 1.68 ± 0.59, and 1.70 ± 0.50 (mean ± standard deviation) m/s, respectively. These results indicate that the hardness of the uterine corpus changed over time, whereas that of the uterine cervix was not significantly altered ($p=0.44\times10^{-10}$ for the corpus and $p=0.06$ for the cervix). The uterine corpus had a significantly higher hardness than the uterine cervix at each of the four time points examined ($p<0.05$).

Discussions: Our results suggest that ARFI is useful to assess the uterine involution as the numerical value. Further, ARFI may be used for diagnosis of cervical ripening during pregnancy using transvaginal probe, when the fetal safety has been established.

S-118

Adverse Outcomes in Twin Pregnancies Complicated by Vaginal Bleeding. Jessica A McPherson, Anthony O Odibo, Anthony L Shanks, Kimberly A Roehl, George A Macones, Alison G Cahill. *Obstetrics & Gynecology, Washington University in St. Louis, St. Louis, MO, USA.*

Objective: As common as bleeding in pregnancy is, no evidence exists regarding risks associated with vaginal bleeding in the first half of a twin pregnancy. The objective of this study was to estimate the risks of adverse outcomes associated with vaginal bleeding in twin pregnancies.

Methods: In a retrospective cohort study of all consecutive twin pregnancies undergoing anatomic survey (gestational ages 17 to 22 weeks) between 1990 and 2008, we compared women who reported bleeding in the first half of pregnancy (< 22 weeks) to those who did not. Assessment of bleeding was done prior to completing the anatomic survey. Exclusion criteria included monoamniotic pregnancy, twin-to-twin transfusion syndrome, and placenta previa. Primary outcomes included pregnancy induced hypertension, abruption, preterm premature rupture of membranes (PPROM), preterm birth (PTB) before 34 weeks, and intrauterine growth restriction (IUGR). Logistic regression analysis was used to estimate the risk of each outcome.

Results: Of 2445 twin pregnancies, 2231 were available for study after exclusion criteria. 2153 (96.5%) had outcome information available and were included in the final analysis. One hundred eighty (8.4%) women reported bleeding in the first half of pregnancy. Twin pregnancies with vaginal bleeding had significantly higher risks of abruption, PPROM, and PTB compared to twin pregnancies without bleeding, which remained significant after adjusting for gestational week at delivery, history of preterm birth, chorionicity, nulliparity, African American race, and body mass index >30 kg/m^2.

Adverse Outcomes after Vaginal Bleeding

Outcome	Bleeding n=180	No Bleeding n=1973	Unadjusted Relative Risk (95% CI)	Adjusted Odds Ratio (95% CI)	p-value
Pregnancy Induced Hypertension	24.4%	19.8%	1.24 (0.94-1.62)	1.26 (0.88-1.81)	0.21
Abruption	7.8%	1.2%	6.67 (3.50-12.74)	10.47 (8.68-12.63)	<0.05
PPROM	20.6%	10.0%	2.06 (1.50-2.82)	2.73 (2.38-3.13)	<0.05
Preterm Birth (<34 weeks)	31.7%	22.7%	1.40 (1.07-1.82)	1.77 (1.22-2.57)	<0.05
Any IUGRα	17.5%	22.3%	0.78 (0.53-1.16)	0.82 (0.51-1.32)	0.42

α Growth <10th% on Alexander Curve

Conclusion: Our results suggest twin pregnancies complicated by vaginal bleeding prior to anatomic survey have an increased risk of abruption, PPROM, and PTB over the background risk conferred by carrying twins.

S-119

Pregnancy in Women with Repaired Coarctation and Bicuspid Aortic Valve. Torri D Metz,[1] Marc Jackson,[1] Anji T Yetman.[2] *[1]Obstetrics and Gynecology, University of Utah; [2]Pediatrics, University of Utah.*

Objective: Both congenital aortic coarctation (CoA) and bicuspid aortic valve (BAV) are manifestations of a diffuse arteriopathy. Approximately 30-40% of patients with CoA also have BAV. There are little data on pregnancy outcomes in women with both lesions. We sought to assess cardiac and reproductive outcomes in women with repaired CoA and BAV.

Study Design: Obstetrical and cardiac records of all reproductive aged women with a diagnosis of CoA who were followed at three tertiary care centers over the past 5 years were reviewed. Patients with an unrepaired CoA, trileaflet aortic valve, or genetic syndrome were excluded. Serial echocardiogram data were reviewed for longitudinal changes in aortic valve function, aortic obstruction or aneurysm formation over time.

Results: Of 49 women with repaired CoA and BAV, 26 had a total of 99 pregnancies (median 3, range 1-14) resulting in 76 live births. 4 women had only one pregnancy. The spontaneous abortion rate was 25% and the stillbirth rate was 2%. Of the 48 liveborns with detailed delivery data, 38% were delivered via cesarean and 23% were preterm. The mean gestational age at delivery was 37.8±2.8 wks. Pregnancy complications included hypertension in 28% and preeclampsia in 13%. One patient developed CHF antepartum. There were no maternal or neonatal deaths. Congenital heart disease (CHD) requiring surgical intervention was present in 9% of offspring.

Women were followed for 7.2±5.0 years after pregnancy and had a mean maternal age of 24.0±4.3 years. Long-term maternal adverse cardiac complications were compared to a group of 23 nulliparous controls with CoA and BAV who were similar for age (p=0.74). A composite adverse outcome (including recurrent coarctation, aortic aneurysm, dissection, aortic valve dysfunction requiring intervention, or heart failure) occurred much more commonly in women with a prior pregnancy (54 vs 12%, p<0.001).

Conclusions: Women with repaired CoA and BAV can tolerate pregnancy without clinical decompensation but should be counseled as to the high incidence of CHD in offspring and increased long-term cardiovascular risks.

S-120

Perinatal Outcome, Social and Financial Impact of African Refugees Delivered in a Tertiary Hospital in Tel-Aviv, Israel. Nadav Michaan, Yaron Gil, Sagi Amzalag, Ziv Tzafrir, Joseph Lessing, Ariel Many. *Obstetrics & Gynecology, Lis Maternity Hospital, Tel-Aviv Medical Center, Sackler School of Medicine, Tel-Aviv, Israel.*

Background: 25,000 refugees from Eritrea and Sudan reside in Israel. These refugees escape ethnic persecution and reach Israel by foot after crossing the Egyptian border. In the L&D ward, communication with these refugees is limited and pregnancy follow-up is usually absent.

Objective: To describe the magnitude of this phenomenon and to compare perinatal outcome between refugees and Israeli parturients.

Methods: Medical and financial records of all refugees delivered between May 2010 and May 2011 were retrieved. Perinatal outcome was compared to non-refugee controls.

Results: 254 refugees were delivered during this period (2.3% of all deliveries) including 7 terminations of pregnancies in women who were raped during their escape. Refugee parturients were significantly younger, leaner and with more primiparas. Demographics are shown in table 1. Premature deliveries before 37 weeks were 2.4 times more common in refugees (9.3% vs. 4%, P=0.02, CI 1.08-5.6). These numbers remained significant for deliveries under 34 weeks (3.6% vs. 0.8%, P=0.036). Refugees were 27.4 times more likely to undergo urgent cesarean sections (97% vs. 53%, P<0.0001, CI 3.5-582) and neonates were 3.1 times more likely to require NICU admission (6.1% vs. 2.0%, P=0.023, CI 1.05-10.1). Moreover, refugees had significantly more cases of meconium and episiotomies while receiving less epidural analgesia. There were 2 IUFD cases among refugees compared to 13 out of 11239 deliveries during this time period (0.81% vs. 0.11%, P=0.036). 68% of refugees had medical fees outstanding with a total debt of $738,000.

Demographic Characteristics

	Cases	Controls	P Value
Mean Age	25.9	32.5	<0.0001
Primigravida	126	83	0.0002
Primipara	137	109	0.0016
Mean Gestational Age (weeks)	39	39	NS
Prematurity<37 weeks	23	10	0.02
Prematurity<34 weeks	9	2	0.036
Post Date>42 weeks	19	3	0.0004
BMI[1] before pregnancy	22.1	22.1	NS
BMI [2] during delivery	25.8	27.2	0.036
BMI[2]-BMI[1]	3.9	5.4	0.016

Conclusions: The phenomenon of African refugees delivered at our center is of unprecedented magnitude with both medical and ethical implications. These parturients proved susceptible to adverse perinatal outcomes compared to their Israeli counterparts. Setting a pregnancy follow-up plant could prevent these adverse outcomes and, in the long run, reduce financial costs involved with treatment of this population.

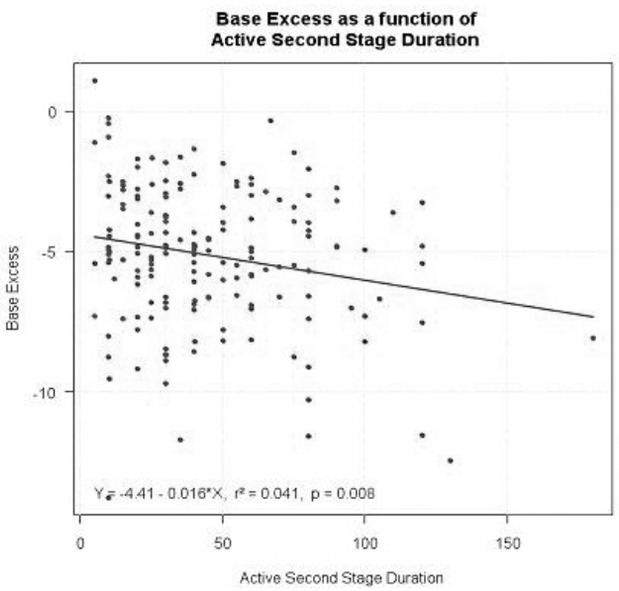
S-121

Effect of Oxytocin during Labor on Umbilical Artery PH. Serena Mussi,[1] Maddalena Incerti,[1] Anna Locatelli,[1] Federica Accordino,[1] Sara Consonni,[1] Alessandro Ghidini,[2] John C Pezzullo.[3] [1]*Obstetrics and Gynecology, S. Gerardo Hospital, Monza, Italy;* [2]*Perinatal Diagnostic Center, Inova Alexandria Hospital;* [3]*Department of Statistics, Georgetown University Medical Center, Washington.*

OBJECTIVE: The presence of tachysytole is associated with the use of oxytocin during labor and low pH value at birth, but its correlation with FHR tracing has not been evaluated. The aim of the study was to assess the effect of oxytocin and tachysystole on umbilical artery (UA) pH.

STUDY DESIGN: This is a prospective observational study of all term pregnancies with a singleton fetus in cephalic presentation which received oxytocin for induction or augmentation of labor and delivered from February 1st until May 31st 2011 in our Department. Cases ending in cesarean delivery before the II stage of labor were excluded. The management of labor was standardized in all cases. Fetal heart rate (FHR) tracings were prospectively classified using ACOG classification. Independent correlators of UA pH were identified using multivariate linear regression with P< 0.05 considered significant.

RESULTS: 210/808 (25.9%) women received oxytocin during the study period, and 110 of them (52.4%) had spontaneous onset of labor. Maximum dose of oxytocin was 13.5 ± 10.0 mU/min. Mean duration of labor was 367.7 ± 194.4 min and duration of active II stage (pushing efforts) was 45.2 ± 30.6 min. Abnormal FHR tracing was diagnosed in 17.4% and tachysystole during the II stage of labor in 51%. At linear regression analysis, abnormal FHR tracing and duration of active II stage were independently correlated with UA pH, whereas maximum dose of oxytocin and tachysystole were not. The duration of active II stage was significantly correlated with UA Ph (p= 0.031), Base Excess (BE, p= 0.008) and lactate (p < 0.001) at birth so that every hour of maternal pushing efforts decreases the BE by 1.0 ± 0.4 mmol/l (figure).



CONCLUSION: In patients receiving oxytocin, the duration of active II stage of labor and abnormal FHR tracings correlate with pH, BE and lactate, whereas use of oxytocin (even when associated with tachysystole) does not.

S-122

VBAC Success in High Risk Women. Jodi Regan, Katherine Wolfe, Candice Snyder, Emily DeFranco. *Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*

OBJECTIVE: The March 2010 NIH Consensus Development Conference Statement on VBAC identified several critical knowledge gaps in clinical decision making regarding trial of labor versus repeat cesarean in women with a prior cesarean. The aim of this study is identify factors associated with VBAC success in high risk women.

STUDY DESIGN: We performed a population-based retrospective cohort study of all births in the state of Ohio in 2006 & 2007 to evaluate factors associated with an increased likelihood of successful VBAC in high risk women. We defined the high risk population as singleton gestations in women with one previous cesarean who had ≥1 of the following high risk factors: BMI≥30, hypertension or diabetes. We stratified factors into 4 groups: demographic, social, medical, and prenatal care, and compared them between high risk women with successful vs failed VBAC attempt. Multivariate logistic regression estimated the relative influence of each factor on successful VBAC.

RESULTS: There were 291,782 births analyzed.Of those, 79,084(27.1%) were in high risk women. 8658(10.9%) had one previous cesarean; 1,433(16.6%) had a trial of labor after cesarean. Of those 974(70%) had a successful VBAC and 459(30%) had a failed TOL. Factors significantly associated with VBAC success in high risk women are: prior vaginal delivery, pregnancy weight gain ≤30 lbs, Caucasian race, and labor augmentation. These factors were NOT associated with successful VBAC in high risk women: maternal age, marital status, educational status, medical insurance type, cigarette use, birth weight, gestational age, or type of delivering obstetric care provider.

CONCLUSION: High risk women with one prior cesarean are unlikely to undergo a TOL after cesarean, but have a similar rate of VBAC success as other populations. This information may assist obstetric care providers to identify the optimal population of high risk women in whom to encourage VBAC.

Predictors of VBAC Success in High Risk Women

	VBAC n=974	Failed TOL n=459	aRR (95% CI)
Prior VD	557 (60.0)	157 (35.3)	2.55 (1.99-3.26)
Labor augmentation	229 (23.6)	70 (15.3)	1.59 (1.15-2.20)
Caucasian	652 (66.9)	292 (63.9)	1.31 (1.01-1.69)
Pregnancy weight gain <= 30 pounds	616 (68.8)	254 (61.2)	1.30 (1.01-1.68)

Dichotomous variables expressed as Number (percent) Continuous variables expressed as Mean (+/- standard deviation) VBAC=vaginal birth after cesarean, TOL=trial of labor, VD=vaginal delivery

S-123

Significant Variability in Antenatal Detection of Small-for-Gestational Age Neonates among Tertiary Centers. Adam T Sandlin,[1] Everett F Magann,[1] Songthip T Ounpraseuth,[1] Josh D Dahlke,[2] Elena Igwe,[3] Eugene Chang,[4] Alfred Z Abuhamad,[5] Suneet P Chauhan.[5] [1]*Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA;* [2]*Obstetrics and Gynecology, Naval Medical Center Portsmouth, Portsmouth, VA, USA;* [3]*Obstetrics and Gynecology, Temple University, Philadelphia, PA, USA;* [4]*Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, USA;* [5]*Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA, USA.*

Objective: To determine the antenatal detection rate of small-for-gestational age (SGA) neonates among 4 tertiary centers and identify factors between the centers which may influence the detection rates.

Methods: All non-anomalous singletons, with sonographic exam before 22 weeks and SGA (birth weight < 10% for Gestational Age) delivered in 2009 at 4 tertiary centers were identified. If IUGR was detected antenatally, SGA was considered detected. Patient's demographic variables, risk measures and infant's birth characteristics were assessed. Comparative analysis was performed with p-values < 0.05 considered statistically significant.

Results: In 2009, there were 11,487 births and 8% (929) were SGA that met the inclusion criteria. The antenatal detection rate of SGA was 25%. The detection rates (18-36%, p<0.001) differed among the centers. Center I differed from Centers III/IV (p<0.0005) in SGA detection. Between centers, there was a difference in ethnicity (p<0.0001), nulliparity (p=0.039), and BMI at first visit (p<0.0002) and a difference in the following risk factors: preterm labor (p<0.0001), PROM (p<0.0001), cigarette use (p=0.0035), pre-gestational diabetes (p=0.0034), gestational age at delivery (p=0.001), and composite risk (p=0.0004). No difference was seen in birth weight (<5% or 5-9%), BMI at delivery, or in the risk factors of 2nd/3rd trimester bleeding or hypertensive disease. There was a difference between Center I vs. Centers II/III/IV and Center II vs. Centers III/IV in the frequency of sonographic estimation of fetal weight (SEFW) within 4 weeks of delivery (p<0.0001).

Conclusion: Significant variation in SGA detection exists between centers. There are differences in several variables and risk factors linked with SGA detection between centers. Further study is warranted to investigate whether increasing the frequency of 3rd trimester SEFW would improve the detection rate of SGA and decrease the variation in SGA detection.

S-124

Can Abdominal Circumference in the Second Trimester Predict the Large for Gestational Age Neonate? Rachelle A Schwartz, Lena Hachem, Barak Rosenn. *Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, New York, NY, USA.*

Objective: The goal of this study was to determine if a fetal abdominal circumference (AC) ≥90%, measured in the second trimester, is a risk factor for the development of a large for gestational age (LGA) neonate.

Study Design: A case-control study was conducted on all women with a fetal AC≥90%, as determined by ultrasound between 18-24 weeks gestation, from 2008-2010. Control subjects (patients with an AC between 10th -89th %, determined within the same gestational age period), were randomly selected in a 1:1 ratio. Additional inclusion criteria included: singleton pregnancy, well defined estimated date of confinement, documented birth weight, and delivery at ≥37 weeks gestation. LGA was defined as birth weight ≥90% for the gestational age at birth. The sensitivity, specificity, positive and negative predictive values, and likelihood ratio of an AC ≥90%, for the development of LGA, were calculated. Statistical analysis was performed using Chi square and Student's t-test.

Results: 372 cases and 372 controls were analyzed. Cases were significantly different from controls in regard to birth weight (3670+/-454g vs 3367+/-452g, p<0.001), mean AC (93+/-2.5% vs 43+/-19.7%, p<0.001) and multiparity (45%vs 67%, p<0.001), respectively. There were no differences in gestational age at birth (39.4+/-1.1wks vs 39.3+/-1.2wks, p=0.2) or GDM (8.6% vs 7.3%, p=0.3).The crude and adjusted (for multiparity, GDM and post-EDC) odds ratios for the occurrence of LGA in fetuses with an AC ≥90% in the second trimester, are listed in Table 1. The ability of a fetal AC≥90%, in the second trimester, to predict LGA at birth was as follows, sensitivity 74%, specificity 57%, positive predictive value 32%, negative predictive value 89%, and likelihood ratio 1.7.

Conclusions: An abdominal circumference >90%, in the second trimester, is an independent risk factor for the development of an LGA neonate.

Table 1. LGA Risk when AC ≥90%

	Odds Ratio	95%CI	P
LGA	3.75	[2.54-5.55]	<0.001
Adjusted			
Multiparity	4.74	[3.34-6.71]	<0.001
Gestational Diabetes	3.84	[2.93-5.15]	<0.001
≥40 weeks	4.10	[2.99-5.62]	<0.001

S-125

Male Sex and the Risk of Perinatal Death. Gordon CS Smith. *Department of Obstetrics and Gynaecology, University of Cambridge, Cambridge, United Kingdom.*

Background

Previous studies have demonstrated associations between male sex and a number of adverse pregnancy outcomes. However, there is no systematic analysis, to our knowledge, which addresses (1) the nature of the association between male sex and different causes of stillbirth and neonatal death, and (2) the proportion of losses that can be attributed to the excess risk of death among males.

Methods

We analysed records from 1992-2008 in the linked database of pregnancy and perinatal death data for Scotland. We included singleton births from 24-43 weeks gestation with a birth weight >399g. Cause of death was sub-divided by a modification of the Wigglesworth classification system. Analysis was by relative risk (95% CI, P) for males, referent to females. Multivariate analysis was performed using logistic regression.

Results

There were records for a total of 932,375 births, including 4,648 stillbirths and 2,331 neonatal deaths. The risk of death was 5.5 per 1,000 among females and 6.2 per 1000 among males (RR for males 1.14, 95% CI 1.08-1.20, P<0.001). Male sex was associated with an increased risk of perinatal death due to fetal anomaly (1.18, 1.06-1.31, P=0.003), antepartum stillbirth due to abruption (1.51, 1.27-1.78, P<0.001), antepartum stillbirth where the baby was small for gestational age (1.29, 1.06-1.59, P=0.01) and neonatal death without anomaly (1.20, 1.09-1.33, P<0.001). There were no significant associations with stillbirth due to pre-eclampsia or other causes, including intrapartum stillbirth. The risk of neonatal death was no longer significant after adjustment for gestational age at birth, indicating that the association was mediated by the increased risk of preterm birth among males. When analysed by cause of preterm birth, male sex was associated with an increased risk of spontaneous preterm birth (1.21, 1.18-1.23, P<0.001) and preterm birth with premature rupture of the membranes (1.23, 1.16-1.31, P<0.001), but was not associated with medically indicated preterm delivery (1.02, 0.98-1.06, P=0.24). Overall, 5.4% of stillbirths and 10.3% of neonatal deaths were attributed to the increased risk among males.

Conclusions

Male sex is associated with an increased risk of perinatal loss due to anomalies, placental abruption, poor fetal growth, spontaneous preterm birth and preterm premature rupture of the fetal membranes. Despite the significant contribution to overall rates of perinatal mortality, the biological basis for these differences remains obscure.

S-126

Obstetric Outcomes in Twin Pregnancies with Fibroids. Molly J Stout, Anthony O Odibo, Anthony L Shanks, Ryan Longman, George A Macones, Alison G Cahill. *Obstetrics and Gynecology Division of Maternal Fetal Medicine, Washington University in Saint Louis, Saint Louis, MO, USA.*

Objective: To estimate the association of adverse outcomes in twin pregnancies with fibroids present at second trimester anatomic survey.

Study Design: A retrospective cohort study of all consecutive women with twin pregnancies who had routine second trimester ultrasound anatomic survey between 17-22 weeks at a large, tertiary care center. The presence or absence of fibroids was noted at routine second trimester ultrasound. Data on maternal socio-demographics and medical history were obtained from patient history and prenatal records. Obstetric outcomes were collected prospectively as pregnancies progressed. Twin pregnancies complicated by fibroids were compared to twin pregnancies without fibroids. Primary outcomes were: placenta previa, placental abruption, intrauterine growth restriction (IUGR), growth discordance, preterm premature rupture of membranes(PPROM), and preterm birth (PTB) less than 37, 34, 28 & 24 weeks. Univariable and multivariable analyses were performed. A subgroup analysis of women with large fibroids (≥6cm) was performed.

Results: Of 2,445 twin pregnancies, fibroid incidence was 2.3% (n=56 pregnancies). A total of 52 fibroids < 6cm, and 7 fibroids ≥ 6cm were present in the cohort. Women with twin pregnancies and 1 or more fibroids were no more likely to have placenta previa, placental abruption, intrauterine growth restriction, growth discordance, intrauterine fetal death or delivery at early gestational ages than women with twin pregnancies with no fibroids.

Outcome	Fibroid (n=56)	No Fibroid (n=2445)	Relative Risk (95%CI)
Placenta Previa (n=17)	0%	0.8%	NA
Abruption (n=42)	1.9%	1.9%	1.0 (0.1-7.3)
IUGR (n=331)	19.4%	22.1%	0.9 (0.4-1.7)
Discordant Growth (n=57)	2.8%	3.5%	0.8 (0.1-5.7)
PPROM (n=257)	5.7%	11.6%	0.5 (0.2-1.5)
PTB < 37 weeks (n=1528)	71.4%	62.3%	1.1(a)(1.0-1.4)
PTB <34 weeks (n=588)	25.0%	24.0%	1.0 (b) (0.7-1.6)
PTB <28 weeks (n=166	7.1%	6.7%	1.0 (0.4-2.7)
PTB <24 weeks (n=84)	3.8%	3.4%	1.0 (0.3-4.1)

a) adjusted odds ratio 1.2 (0.7-2.3) adjusted for parity, tobacco, prior PTB. b) Adjusted odds ratio 1.0 (0.5-1.9) adjusted for PTB

Conclusion: Contrary to recent publications suggesting that women with singleton pregnancies and fibroids are at increased risk for adverse pregnancy outcomes, the increased risk for adverse pregnancy outcomes inherent in twin pregnancies does not appear to be increased by the presence of fibroids.

S-127

The Analysis of 303 Cases with Massive Postpartum Hemorrhage. Naoaki Tamura, Hiroaki Itoh, Toshiyuki Uchida, Kazunao Suzuki, Naohiro Kanayama. *Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Objective; Postpartum hemorrhage (PPH) is a critical event, which sometimes leads to maternal death. In Japan, 4 major academic groups including Japan Society of obstetrics and Gynecology defined the more than 2000 ml hemorrhage as a life threatened PPH in April 2011. Various predisposing factors of PPH such as trauma and coagulation defects have been reported; however, it has been remain to be clarified the actual causes of PPH. In this study, we analyzed clinical courses and causative complications of the cases of life threatened PPH in Japan. ***Methods;*** 303 cases of more than 2000 ml PPH were enrolled and retrospectively assessed by using a questionnaire survey composed of following questions, maternal age, gravida and parity, maternal complications, gestation period, mode of delivery, volume of hemorrhage, the shock index during management, evidence of blood transfusion and total hysterectomy, physician's diagnosis including trauma, and others. ***Result;*** Age

(mean ± SD) was 34 ± 4.88 yo. Gravida and parity (mean ± SD) were 1.17 ± 1.43 and 0.78 ± 1.07, respectively. As complications, 59 cases with placenta previa, 11 cases with preeclampsia and 8 cases with gestational diabetes mellitus were presented. Gestation period (mean ± SD) was 35.91 ± 4.34 weeks. 46 cases of vaginal delivery and 257 cases of Caesarean section were performed. Hemorrhage volume (mean ± SD) was 2920 ± 1512 ml. The lowest shock index during management (mean ± SD) was 1.0 ± 0.37. 101 cases were received blood transfusion and 32 cases were performed total hysterectomy. Physician's diagnosis were 185 cases (61 %) of uterine atony, 39 cases (12 %) of placenta accreta due to placenta previa and elsewhere, 8 cases (2.6 %) of placenta abruption and 2 cases of trauma such as cervical laceration. There was no fatal case. ***Conclusion;*** Our study demonstrated the main cause of PPH was uterine atony. Uterin atony should be one of observations of postpartum uterus caused by several factors. The further detail investigation of individual cases of uterine atony will give us a clue to understand pathophysiology of PPH.

S-128

The Combination of Maternal Hypothyroidism and Diabetes Mellitus – A Risky Dual Gestational Endocrinopathy. Dan Tirosh,[1] Neta Benshalom-Tirosh,[1] Lena Novack,[2] Fernanda Press,[1] Ruthy Beer-Weisel,[1] Arnon Wiznitzer,[1] Moshe Mazor,[1] Offer Erez.[1] *[1]Obstetrics and Gynecology, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of Negev, Beer-Sheva, Israel; [2]Department of Epidemiology and Health Services Evaluation, Faculty of Health Sciences, Ben-Gurion University of Negev, Beer-Sheva, Israel.*

Objective: Maternal diabetes mellitus (DM) and hypothyroidism are each associated with increased rate of pregnancy complications; however, their combined morbidity during gestation is poorly studied. The aims of this study were to determine the prevalence of DM & hypothyroidism and whether this combination is associated with adverse maternal and neonatal outcome.

Study design: We conducted a retrospective cohort study of pregnant women (n=232,293 deliveries) including the following groups: 1) hypothyroidism & DM (n=171); 2) hypothyroidism (n=1,502); 3) DM (n=13,324); and 4) pregnant women with neither of the above (n=217,296) that serve as a control group.

Results: The prevalence of DM & hypothyroidism in our population was 0.07%. In comparisons to the other study groups, women with DM & hypothyroidism had a higher rate of infertility (p<0.001), preeclampsia (p<0.001), chronic hypertension (p<0.001), preterm birth (p<0.001), and cesarean deliveries (p<0.001) (see Table). Neonatal mortality and morbidity were comparable among the groups. In GEE model, the combination of DM & hypothyroidism was an independent risk factor for cesarean section (OR 3.46; 95% CI 2.53-4.75).Conclusion: The combination of DM & hypothyroidism is rare, yet it is associated with a higher rate of infertility, cesarean sections, preterm deliveries, and hypertensive disorders of pregnancy. This dual endocrinological combination is an independent risk factor for cesarean section. These findings suggest that such patients are at risk for perinatal complications and should be followed and delivered as high risk pregnancies.

Main clinical characteristics of the study groups

characteristics	Control n=217,296	DM n=13,324	Hypothyroidism n-1,502	Hypothyroidism +DM n=171	p-value
Infertility treatment	4.9%	9.4%	6.3%	11.1%	<0.0001
Preeclampsia	4.2%	7.5%	5.4%	14%	<0.0001
Chronic hypertension	1.2%	6.6%	2.3%	11.1!!	<0.0001
PROM	7.8%	6.7%	11.7%	10.5%	<0.0001
Preterm birth	7.4%	9.7%	8.6%	14%	<0.0001
Cesarean Section	12.3%	27%	23.4%	44.4%	<0.0001

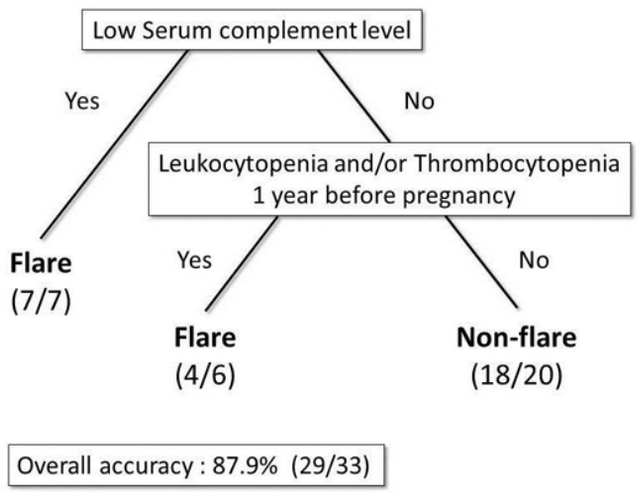
S-129

Predictors of Systemic Lupus Erythematous Flare during Pregnancy. Akihiko Ueda, Eiji Kondo, Junzo Hamanishi, Keiji Tatsumi, Ikuo Konishi. *Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Shogoin Kawahara-cho, Sakyo-ku, Kyoto, Japan.*

Systemic lupus erythematous (SLE) is associated with poor pregnancy outcomes especially when it aggravates during pregnancy. Here we performed a retrospective study to identify risk factors for SLE exacerbation during pregnancy and to evaluate its pathogenesis. The study included 32 patients with SLE (33 pregnancies), managed in our institution between January 2005 and September 2011. All patients met criteria for SLE before pregnancy and were assigned to two groups, a flare group (13 cases) or a non-flare group (20 cases). Flare was defined as new onset or relapse of SLE manifestations which had not been observed at the time of conception. Two groups were analyzed by comparing SLE manifestations, laboratory data, and obstetrical outcomes. Microscopic analyses of placenta were done for 10 flare and 9 non-flare cases available. Statistical analyses were performed using Mann-Whitney test, Chi-squared test or Weka J48 decision tree-inducing algorithm.

The flare group had shorter gestational duration (233 vs 264 days, p<0.01), lower birth weight (S.D. = -1.82 vs -1.25, p=0.02), and higher risk of caesarian section (79 vs 40%, p=0.03). Four factors; no history of discoid lupus (0 vs 35%, p=0.02), presence of leukocytopenia and/or thrombocytopenia before 1 year of pregnancy (62 vs 10%, p<0.01), coexisting antiphospholipid syndrome (23 vs 0%, p=0.02), and low serum complement levels in the early stage of pregnancy (30.1 vs 43.4U/mL, p<0.01), were revealed as independent risk factors for SLE flare. Weka J48 software determines a decision tree using the minimum number of factors. Among four factors, two were selected to draw a decision tree (figure). Analyses of placental pathology showed that acute atherosis (60 vs 0%, p<0.01) and infarction (80 vs 33%, p=0.04) were frequently observed in the flare group.

This study indicated that previous SLE manifestations and serum complement factor levels are good predictors for SLE exacerbation during pregnancy. Poor obstetrical outcome may be attributed to impaired blood circulation on the maternal side of the placenta.



S-130

Pregnancy Outcome Following History Indicated Abdominal Cerclage. Sophia NE Webster,[1] Nicholas M Wales,[2] Vasso Terzidou,[2,3] Philip J Steer.[3] *[1]Obstetrics and Gynaecology, Royal Victoria Infirmary, Newcastle Upon Tyne, United Kingdom; [2]Obstetrics and Gynaecology, Chelsea and Westminster Hospital, London, United Kingdom; [3]Academic Department of Obstetrics, Imperial College London, London, United Kingdom.*

Introduction

Some women who suffer recurrent midtrimester loss or premature birth are diagnosed with cervical incompetence and some who have had uterine or cervical surgery may also have this condition. Transabdominal cerclage is an alternative to Shirodkar or McDonald cerclage in cases of major cervical deficiency.

Methods

All women who underwent elective abdominal cervical cerclage at the Chelsea and Westminster Hospital between January 2000 and April 2011 were retrospectively identified using theatre logbooks and electronic maternity records. Notes were then reviewed for past history, clinical details, operative procedures and pregnancy outcome.

Results

There were 54 pregnancies in 43 women with abdominal cerclage and for whom outcome data were available. Thirty-two (59%) had pre-pregnancy cerclage and 22 (41%) first trimester cerclage. Fifty-two sutures were inserted through mini laparotomy and 2 laparoscopically with a low complication rate. Thirty-five (65%) gave a history of at least one midtrimester loss or very preterm birth (<28 weeks). The remainder had undergone previous cervical or uterine surgery likely to impair cervical function. There were 47 livebirths, 1 ectopic pregnancy, 2 miscarriages <14 weeks managed by uncomplicated uterine evacuation with cerclage left in situ, 3 terminations of pregnancy (1 for trisomy and 2 for premature rupture of membranes (PROM) <20 weeks) and 1 stillbirth. Both cases of PROM underwent cerclage removal and induction. Of the livebirths, 42 (78%) reached 37 weeks. All were delivered by caesarean section with a low complication rate. Birthweights were as expected for the

gestational age distribution. None of the birth outcomes or complications were significantly different between women undergoing pre-pregnancy or women undergoing first trimester cerclage although there was a tendency to more births <37 weeks in the latter group.

Discussion

Our series demonstrates a favourable outcome following abdominal cerclage with low complication rates. Even in cases of major cervical deficiency, with this intervention the chances of a successful pregnancy outcome are good. There remains a necessity to clarify the indication for the procedure and to discover its true potential benefit in a randomized control trial setting.

S-131

Polymorphisms in the Microsomal Epoxide Hydrolase Gene and the Risk of Preeclampsia: A Meta-Analysis. Shu-Qin Wei,[1] Hui-Ping Qi,[1] Pierre Julien,[2] William D Fraser.[1] *[1]Obstetrics and Gynecology, Sainte-Justine Hospital, University of Montreal, Montreal, QC, Canada; [2]Lipid Research Center, Laval University Medical Center (CHUQ).*

OBJECTIVE:

Microsomal epoxide hydrolase (EPHX) plays an important role in both detoxification processes and repair following oxidative stress. The Tyr113His polymorphism in exon 3 and the His139Arg polymorphism in exon 4 of the EPHX gene have been reported to be associated with variations in EPHX activity. The aim of this study was to determine whether in EPHX genetic variability contributes to individual differences in susceptibility to the development of preeclampsia.

METHODS:

Electronic searches for all publications in databases PubMed and EMBASE were conducted on the associations between EPHX polymorphisms and risk for preeclampsia until September 2011. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated using fixed and random effects models.

RESULTS:

Four studies were included with a total of 1,342 individuals, involving 724 preeclampsia and 618 normotensive controls. This meta-analysis showed that exon 4 139His →Arg polymorphism was statistically significant associated with decreased risk of preeclampsia (OR: 0.42; 95%CI: 0.18-0.97) (p=0.04). No heterogeneity was detected ($\chi 2 = 0.03$; $I^2 = 0$ %). While The Tyr113His polymorphism in exon of EPHX gene was not significantly statistically associated with increased risk of PE (OR: 1.18; 95%CI: 0.91-1.54) (p=0.21). No evidence of publication bias was observed in this study.

CONCLUSION:

The His139Arg polymorphism in exon 4 of EPHX gene is associated with a decreased risk of preeclampsia. There is no evidence of an association between Tyr113His polymorphism in exon 3 of EPHX gene and preeclampsia.

S-132

Balloon Tamponade in Atonic Bleeding Induces Uterine Contraction: Attempt To Quantify Uterine Stiffness Using Acoustic Radiation Force Impulse Elastography before and after Balloon Tamponade. Takashi Yorifuji, Toshitaka Tanaka, Shintaro Makino, Taro Koshiishi, Motoi Sugimura, Satoru Takeda. *Obstetrics and Gynecology, Juntendo University School of Medicine, Hongo Bunkyo-ku, Tokyo, Japan.*

Introduction: Balloon tamponade can be performed rapidly and easily, and is highly effective for achieving hemostasis in atonic bleeding. However, the mechanisms underlying hemostasis have not been elucidated. We have performed balloon tamponade in six women with atonic bleeding after normal vaginal delivery, and complete hemostasis was achieved with the balloon alone. A metreurynter was inserted into the uterine cervix as a balloon catheter and inflated with enough saline both to prevent the device falling into the vagina and to stop the bleeding from the uterine os. In each of the six women, the placenta was located in the uterine corpus, or its separation surface covered the uterine corpus. Although the balloon was placed in the uterine cervix, we could palpate good uterine contractions after inserting the balloon, suggesting a hemostatic mechanism whereby balloon insertion into the uterine cervix induces contraction of the uterine corpus. We estimated the stiffness of the uterine corpus and cervix by acoustic radiation force impulse elastography before and after balloon tamponade in one patient. **Methods:** We used a Siemens ACUSON S2000 US system (Mochida Siemens Medical Systems Co., Ltd, Tokyo, Japan). An acoustic push pulse was transmitted to the uterine corpus and cervix at a depth of approximately 15mm from the uterine surface, where the shear-wave velocity was calculated and expressed with a numerical value (in meters per second). Measurements of myometrial stiffnesswere performed before, immediately after, and 1, 2 and 24hours after balloon insertion. **Results:** Uterine corpus stiffness was 2.79m/s before balloon insertion, 4.09m/s immediately after balloon insertion, 2.60m/s after one hour, 2.35m/s after two hours and 3.34m/s after 24hours. Uterine cervical stiffness was 1.31m/s before balloon insertion, 1.66m/s immediately after balloon insertion, 1.50m/s after one hour, 1.43m/s after two hours and 1.56m/s after 24hours. **Conclusion:** Our findings suggest that the hemostatic mechanisms underlying balloon tamponade in atonic bleeding involve not only direct compression, but also induction of contraction of the uterine corpus by insertion of the balloon into the uterine cervix, leading to hemostasis.

S-133

What Happens after the Puerperium? The Impact of Pregnancy on the Duration of Future Hospitalization. Brett C Young,[1] Britta Panda,[1] Erin Madden,[2] Allison Bryant.[1] *[1]Obstetrics, Massachusetts General Hospital, Boston, MA, USA; [2]Veterans' Research Institute, VCIRE, San Francisco, CA, USA.*

OBJECTIVE: Medical or obstetrical issues may prolong the immediate postpartum hospitalization. It is unknown whether a complicated immediate postpartum hospitalization impacts the duration of subsequent hospitalization between 42 and 365 days postpartum. We sought to determine the relationship between immediate postpartum hospitalization and other obstetrical risk factors that affect the duration of "late postpartum" admissions.

STUDY DESIGN: Data from vital statistics records for all births in California from 1999-2003 were linked with hospital discharge data for hospital admissions from conception to one year post-delivery. For women with a first birth during the study period, the duration of readmission to a hospital between 42 and 365 days post-delivery was calculated. Multivariable logistic regression was used to determine risk factors for these "late postpartum" admissions.

RESULTS: Of 951,528 records reviewed, 1.2% had an admission between 42 and 365 days postpartum. The mean duration of "late postpartum" admission was 3.0 +/- 4.7 days. After adjustment, risk factors that resulted in a longer duration of "late postpartum" admission are included in Table 1. The most common diagnoses at readmission were biliary disease (14.2%), electrolyte abnormalities (3.1%), pancreatitis (3.1%), urinary tract disorders (3.0%) and mood disorders (3.0%).

CONCLUSION: Prolonged hospitalization for the immediate postpartum period, preterm delivery, black race and lack of prenatal care are risk factors for longer hospitalization during the "late postpartum" period which may correspond to the severity of the patient's comorbidities. Identification of patients at risk for prolonged readmission may help providers improve outpatient management of comorbidities and perhaps decrease hospitalization duration.

Risk Factors for duration of "late postpartum readmission" 42-365 days postpartum

	Rate Ratio	95% CI	P-value
Postpartum hospitalization > 4 days	1.13	1.06-1.21	<0.0001
3 days	1.07	101-1.13	0.029
2 days	1.04	0.99-1.10	0.14
1 day	1.00		
Delivery < 28 weeks	1.58	1.36-1.84	<0.0001
28-31 weeks	1.51	1.23-1.85	<0.0001
32-36 weeks	1.06	1.00-1.12	0.054
37+ weeks	1.00		
Maternal age > 40 years	1.26	1.13-1.41	<0.0001
30-39 years	1.05	1.00-1.10	0.055
20-29 years	1.00		
Black race (vs. white)	1.18	1.11-1.26	<0.0001
Hypertension (vs. no hypertension)	1.21	1.13-1.30	<0.0001
No prenatal care (vs. prenatal care)	1.08	1.03-1.14	0.0018

S-134

To Bleed or Not To Bleed: What Are Risk Factors for Early Delivery with Placenta Previa? Brett C Young,[1] Britta Panda,[1] Erin Madden,[2] Allison Bryant.[1] *[1]Maternal Fetal Medicine, Massachusetts General Hospital, Boston, MA, USA; [2]Veteran's Research Institute, VCIRE, San Francisco, CA, USA.*

OBJECTIVE: Placenta previa is an increasingly common condition during pregnancy that coincides with the increasing rate of cesarean deliveries. There are limited data on the incidence and timing of hospitalization as well as pregnancy outcomes once a woman is hospitalized with placenta previa. We sought to determine the incidence and characteristics of women hospitalized with a diagnosis of placenta previa in a large cohort. We additionally examined the association between timing of first hospitalization on the gestational age at delivery.

STUDY DESIGN: Data from vital statistics records for all first births in California between 1999 and 2003 were linked with hospital discharge data. We identified all women carrying a diagnosis of placenta previa who also

had an antepartum hospitalization. Multiple logistic regression was used to determine factors associated with gestational age at delivery, including timing of first hospitalization.

RESULTS: Out of 1,089,678 patient records, 5856 women (0.54%) were diagnosed with placenta previa. 54% of these women had an antepartum admission with a diagnosis of placenta previa. The mean maternal age was 28.7 +/- 7.1 years. The mean gestational age at first antepartum admission was 27.6 +/- 7.2 weeks. 40.6% of women were admitted with placenta previa between 24-32 weeks gestation. 49.1% of women delivered at less than 36 weeks with 12% delivering at less than 32 weeks. After adjustment, hospital admission for placenta previa between 24-28 weeks resulted in a 1.5 week earlier delivery ($p<0.0001$) as compared with admission between 32 and 36 weeks. Women of black race delivered 1.1 weeks earlier ($p=0.0002$) than did white women.

CONCLUSION: Placenta previa is a significant risk factor for antepartum admission and preterm delivery < 36 weeks. Black race and early hospital antepartum admission are risk factors for earlier delivery with placenta previa. These data may be helpful for providers in counseling women with a diagnosis of placenta previa.

S-135

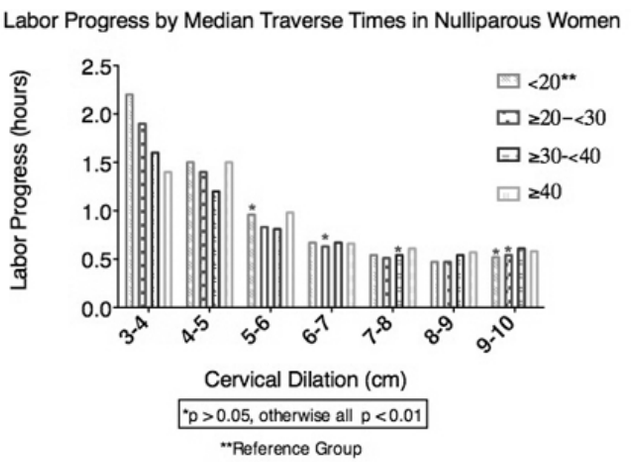
Contemporary Labor Patterns: Does Maternal Age Matter? Mary N Zaki,[1] Juliana Lopez,[2] Weihua Gao,[3] Michelle A Kominiarek,[1] Judith U Hibbard.[1] *[1]Obstetrics and Gynecology, University of Illinois at Chicago; [2]School of Medicine, University of Illinois; [3]CCTS, University of Illinois at Chicago.*

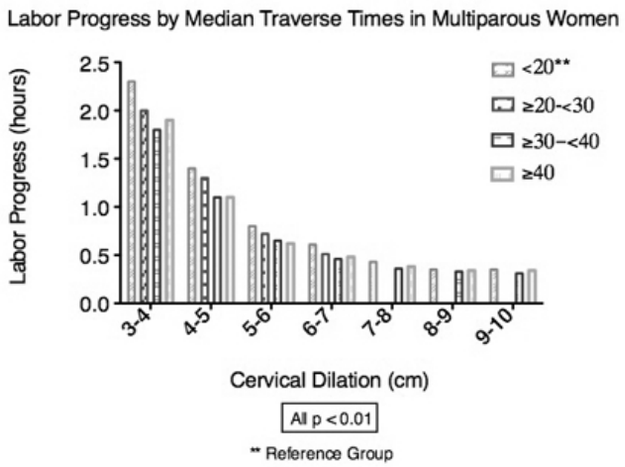
OBJECTIVE: Maternal age at first birth as well as cesarean sections (CS) are increasing in US women. To better understand the labor process in this aging population, we sought to evaluate labor progress and length according to maternal age.

STUDY DESIGN: Data were abstracted from the Consortium on Safe Labor database, a multicenter retrospective study from 19 hospitals in the US. We studied 132,654 laboring gravidas with singleton term cephalic live gestations without a prior CS from 2002-2008. Maternal age categories were <20, ≥20-<30, ≥30-<40 and ≥40 yrs, with the reference group being <20 yrs. Interval-censored regression analysis was used to determine median traverse times of labor (progression cm by cm and 2nd stage length) with 95th%, adjusting for covariates (race, BMI, gestational age, diabetes, induction, augmentation, epidural and birth weight).

RESULTS: CS occurred in 15.3% of women <20 yrs and in 21.9% of those >40 yrs ($P<0.0001$). Nulliparas: Time to progress per cm to 6cm decreased as age increased ($P<0.0001$); length of 2nd stage with or without epidural increased directly with age ($P<0.0001$), and epidural increased the duration of 2nd stage by approximately 30 min in all age groups. Multiparas: Time to progress per cm to 7cm decreased as age increased ($P<0.0001$); 2nd stage with or without epidural was not different among age categories ($P>0.05$), except ≥40 yrs was longer ($P<0.02$).

CONCLUSION: Contemporary labor practices should take into account the changing age profiles of obstetrical populations in the US. Maternal age impacts time spent in 1st stage of labor differently for multiparas and nulliparas. Older nulliparas (≥30 yrs) should be given more time in 2nd stage prior to intervening with CS.





S-136

Doppler Abnormalities in Monochorionic Diamniotic Twin Pregnancies with Discordant Growth. Lisa C Zuckerwise, Unzila Ali, Catalin S Buhimschi, Joshua A Copel, Mert O Bahtiyar. *Ob/Gyn & Reprod Sci., Yale University, New Haven, CT, USA.*

OBJECTIVE: Monochorionic placentation is a significant risk factor for growth discordance. Although discordant growth is more common in monochorionic diamniotic (MCDA) twin gestations complicated by twin-to-twin transfusion syndrome (TTTS), this entity does not account for all cases of growth abnormalities among these twins. This study aims to investigate whether abnormal umbilical artery Doppler velocimetry occurs with higher frequency in MCDA twin gestations characterized by discordant growth when compared to non-discordant twin pairs.

STUDY DESIGN: In a retrospective study design, we analyzed 98 consecutive MCDA twin pregnancies at our institution with an initial ultrasound performed between 2007 and 2010. A 20% difference in birth weight among twin pairs defined discordant growth. Doppler assessment of the fetal circulation was completed with appropriate low-filter and angle corrections. Abnormal Doppler velocimetry was defined as absent or reversed end-diastolic flow in the umbilical artery. In cases complicated by TTTS, we analyzed Doppler data prior to fetal intervention. We employed parametric and non-parametric statistical analysis.

RESULTS: 73 twin pairs met criteria for inclusion, including 16 pairs with discordant growth. There were no differences in maternal age, parity and frequency of assisted reproductive technology. MCDA pregnancies with discordant growth were more likely to be complicated by TTTS (37.5% versus 12.3%, $P=0.02$). Smaller infants in the twin pairs with discordant growth were more likely to have abnormal umbilical artery Doppler velocimetry ($P<0.01$). These findings persisted after TTTS cases were excluded from analysis ($P<0.01$). Abnormal placental cord insertions (marginal or velamentous) occurred more frequently in smaller fetuses with discordant growth ($P=0.03$) and these newborns were more likely to require admission to the neonatal intensive care unit ($P=0.01$).

CONCLUSION: These findings suggest that dysfunctional placentation may contribute to discordant growth as an entity distinct from TTTS.

S-137

Increased Placental Expression of Glucose Transporters in Maternal Obesity. Ometeotl M Acosta, Francesca Gaccioli, Vanessa Ramirez, Donald J Dudley, Theresa L Powell, Thomas Jansson. *Center for Pregnancy and Newborn Research, Dept OB/GYN, University of Texas Health Science Center, San Antonio, TX, USA.*

Introduction: Obese women have an increased risk to deliver a large infant, however the underlying mechanism remains to be fully established. Glucose is the primary energy substrate for the fetus and constitutes a precursor for fatty acid synthesis, which contributes to fetal fat accumulation. Fetal glucose availability is linked to fetal growth by regulating the release of insulin and IGF-I, the two primary fetal growth hormones. We tested the hypothesis that fetal blood glucose levels are elevated in maternal obesity and that the expression of glucose transporters (GLUT) 1 and 9 are increased in the syncytiotrophoblast plasma membranes. **Methods:** Glucose concentrations were determined in paired fasting maternal and fetal samples obtained at cesarean section. We isolated syncytiotrophoblast microvillous (MVM) and

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basal plasma membranes (BM) from placentas of lean women (BMI 21.7±0.8, n=9) who delivered AGA infants (3373±82g) and obese women (BMI 33.7±2.9, n =4) without diabetes who delivered LGA infants (4211±31g) at term. Western blot analysis was used to quantify the expression of GLUT 1 and 9 in MVM and BM. **Results:** Maternal fasting serum glucose levels were not different in obese as compared to lean women. Fetal serum glucose concentrations were slightly higher in infants born to obese mothers (59 ± 4 mg/dl, n = 14) as compared to controls (51 ± 3 mg/dl, n = 7), but this difference did not reach statistical significance (p =0.25). Fetal serum glucose levels were positively correlated to birth weight ($r^2 = 0.2$, n = 21, $p < 0.05$). MVM GLUT 1 expression was unchanged and BM GLUT 1 expression was significantly higher (4.5-fold, $p<0.05$) in obese LGA compared to lean AGA controls. GLUT 9 MVM expression was decreased by 50% (p =0.06) in placentas of LGA babies of obese mothers as compared to lean AGA. GLUT 9 expression in BM appeared to be increased in obese LGA (2.9-fold), however this difference did not reach statistical significance (p =0.12). **Conclusion:** Albeit preliminary, our data are consistent with an up-regulation of glucose transporter expression in BM of obese LGA. The BM is believed to be the rate-limiting step for transplacental glucose transport. We speculate that an increased placental glucose transport capacity contributes to increased fetal glucose concentrations and excess fetal growth in maternal obesity.

S-138

Restricted Nutrient Supply from Mother to Fetus Is Associated with Up-Regulated Placental Amino Acid Transporter and mTOR Protein Expression. Yukiyo Aiko, Eiji Shibata, David J Askew, Toru Hachisuga. *Depertment of Obstetics and Gynecology, University of Occupational and Environmental Health, Japan.*

Background: Preeclampsia (PE) and Fetal Growth Restriction (FGR) are often associated with placental insufficiency, restricted blood flow and reduced nutrient availability. There is evidence that an attempt is made during gestation to normalize fetal growth in this placental environment via regulation of the Amino Acid Transporter (AAT) machinery. However, AAT activity is decreased in FGR compared to uncomplicated pregnancies, suggesting that AAT activity is, in part, directly responsible for the fetal growth phenotype in FGR. The intracellular signaling protein kinase mTOR (mammalian Target of Rapamycin) is responsive to signaling pathways associated with nutrient availability, and is a regulator of AAT activity. We hypothesize that IUGR is a direct result of dis-regulated AAT activity, and is mTOR-dependent.

Material and Methods: To understand AAT activity regulation (or dis-regulation) in FGR we began with a direct analysis of protein expression levels and cellular localization of AAT proteins, and the key AAT regulator, mTOR protein, using immunohistochemistry and Western blotting. In addition, a cohort study of 55 pregnancies examined conditions associated with nutrient restriction during pregnancy, as determined by reduced weight gain, in combination with placental AAT and mTOR protein expression.

Result: Here we report that the expression of type L and Neutral AAT subunits were significantly elevated in the SCT of both PE and FGR-associated placentas compared to normal ($p<0.05$). Total cellular mTOR protein expression was also increased in PE and FGR ($p<0.05$). A positive correlation was identified for reduced weight-gain during pregnancy and placental AAT and mTOR protein expression ($R=0.512$, $P<0.0001$).

Conclusion: These results support the hypothesis that lower nutrient supply from the mother leads to an attempt to normalize fetal growth via increased AAT expression. Furthermore, these results suggest that post-translational regulation of AAT activity is critical to the FGR phenotype.

S-139

Antenatal Dexamethasone Exposure in Mice Does Not Affect Levels of SNAT Proteins in the Placental Microvillous Membrane. Melanie C Audette,[1] Majid Iqbal,[1] John R Challis,[1,4] Rebecca L Jones,[4] Colin P Sibley,[4] Stephen G Matthews.[1,3] *[1]Physiology, University of Toronto, Canada; [2]Obstetrics & Gynecology, University of Toronto, Canada; [3]Medicine, University of Toronto, Canada; [4]Maternal and Fetal Heath Research Centre, University of Manchester, United Kingdom.*

Objective: Synthetic glucocorticoids (sGCs), which are administered to women threatened with preterm labour, differentially regulate the placental system A amino acid transporter in vitro. The system A transporter is composed of three independent proteins (SNAT1, SNAT2 and SNAT4) which are encoded by the Slc38a1, Slc38a2 and Slc38a4 genes. Recently, we demonstrated that sGC treatment administered in mid-gestation reduced murine system A mediated transport at term. This reduction in transporter function was not mediated by altered gene expression, as DEX did not affect Slc38a isoforms (Audette et al. Endocrinol 2011). The molecular mechanisms underlying sGC-induced reductions in system A activity are not known. We hypothesized that maternal sGC treatment down-regulates SNAT protein expression at the placental microvillous membrane in late gestation.

Methods: C57BL/6 pregnant dams were treated with dexamethasone (DEX; 0.1mg/kg, n=6) or saline (n=6) on embryonic day (E)13.5 and E14.5. Placental tissue was collected on E18.5 (term ~E19.5). Protein levels of SNAT1, SNAT2 and SNAT4 (Santa Cruz) were measured in total placental homogenates and in isolated microvillous membrane using western blot. Alkaline phosphatase activity was used to verify enrichment of placental microvillous membrane.

Results: Alkaline phosphatase activity demonstrated similar fold enrichment between vehicle (17.00±1.6) and DEX (20.49±5.5) treated placentae. Protein levels of SNAT1, SNAT2 and SNAT4 were demonstrated in placental homogenates however, were not affected by DEX treatment ($p>0.05$). Similarly, SNAT1, SNAT2 and SNAT4 demonstrated in microvillous membrane vesicles were also unaffected by DEX treatment ($p>0.05$).

Conclusions: Previously we have demonstrated that antenatal sGC treatment in mid-gestation reduces murine placental system A transport prior to term – where reduction in transporter activity is not mediated by alterations in Slc38a gene transcription. Our current results show that sGC treatment does not alter SNAT protein levels in the placenta or microvillous membrane. This suggests that alternative post-translational modifications may reduce transporter function.

S-140

Effects of Sildenafil Citrate on Fetal and Placental Growth in the Placental Specific Igf2 Knockout (P0) Mouse. Mark R Dilworth,[1] Bernadette C Baker,[1] Lewis J Renshall,[1] Philip N Baker,[2] Susan L Greenwood,[1] Mark Wareing,[1] Colin P Sibley.[1] *[1]Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom; [2]Department of Obstetrics and Gynecology, University of Alberta, Canada.*

INTRODUCTION. Sildenafil citrate (SC, Viagra™), a selective and potent inhibitor of phosphodiesterase-5, promotes nitric oxide-dependent vasorelaxation. SC enhances fetal growth and amino acid availability in a sheep model of fetal growth restriction (FGR) [1]. A recent non-randomised clinical trial in pregnancies with severe early-onset FGR suggested that SC improved growth velocity [2]. We tested the hypothesis that SC could increase fetal growth in a mouse model of FGR that demonstrates an abnormal placental exchange barrier phenotype, the placental specific *Igf2* knockout (P0) mouse [3,4].

METHODS. Female wild type (WT) C57Bl6/J mice were mated with P0 males. SC was given via drinking water from E12.5 at 0 (controls, N=8) 0.2 (SC low, N=8) or 0.4mg/ml (SC high, N=9). Fetal and placental weights were measured at E18.5. Maternofetal clearance (K_{mf} ul/min/g placenta) of 14C-MeAIB, a substrate for system A amino acid transport, was estimated following administration to the dam and measurement of fetal radioactivity counts.

RESULTS. Analysis showed that placental weight was both genotype and drug dependent (2-way ANOVA). To explore this further, placental and fetal weights were calculated as a ratio of P0/WT in each litter (expressed as % of WT, median, IQR). Relative placental weight was increased in P0 in SC high (76%, 72-81) compared with control (69%, 67-73) and SC low (70%, 65-74) groups ($P < 0.05$, Kruskal Wallis with Dunn's post-hoc test). There was a trend towards increased fetal weight in SC high (82%, 78-88) compared with control (77%, 76-80) but this failed to reach statistical significance ($P = 0.06$, Kruskal-Wallis). There was no significant difference in $^{MeAIB}K_{mf}$ between groups.

CONCLUSIONS. 0.4 mg/ml SC ameliorates the effect of P0 knockout on placental and, more subtly, fetal weight without altering growth of the WT placenta or fetus. We speculate that SC enhances uteroplacental blood flow sufficiently to improve growth in the P0 conceptus even though the major placental phenotype in this mouse is an abnormally functioning exchange barrier [4].

[1] Satterfield et al 2010. *J Nutr*. 140(2), 251-8.
[2] Von Dadelszen et al 2011. *BJOG*. 118(5). 624-8.
[3] Constancia et al 2002. *Nature*. 417, 945-8.
[4] Kusinski et al 2011 *Placenta*. In press.

S-141

Placental System A and System L Amino Acid Transporter Activity and Expression in Lean and Overweight/Obese Hispanic Women. Francesca Gaccioli, Thomas Jansson, Theresa L Powell. *Dept of Ob/Gyn, Center for Pregnancy and Newborn Research, University of Texas Health Science Center, San Antonio, TX, USA.*

OBJECTIVES: Obese women have an increased risk to deliver a large-for-gestational age baby and enhanced placental nutrient transport capacity may contribute to fetal overgrowth in these pregnancies. Mammalian target of rapamycin (mTOR) is a positive regulator of placental amino acid transport. We previously demonstrated that the phosphorylation of 4E-binding protein 1 (4E-BP1) is increased in placentas of overweight/obese Hispanic women, suggesting an activation of the mTOR signaling pathway. We tested the hypothesis that placental System A and System L amino acid transporter activity and expression are increased in overweight and obese pregnancies.

METHODS: We collected placentas at term from predominantly Hispanic women. Syncytiotrophoblast microvillous plasma membranes (MVM) and basal plasma membranes (BM) were isolated from 27 normal pregnancies, 10 women with normal BMI (21.5 ± 0.7; BW: 3345 ± 79 g, placental weight: 648 ± 27 g) and 17 women with high BMI (33.4 ± 1.4; BW: 3716 ± 120 g, placental weight: 778 ± 53 g). We measured the activity of System A and System L in the MVM vesicles and the protein expression of LAT1 isoform was determined by Western blot analysis.

RESULTS: The activity of MVM System A did not differ between control (50.1 ± 6.2 pmol/mg x 30sec) and high BMI group (45.7 ± 5.1 pmol/mg x 30sec). Similarly, System L transporter activity was not significantly altered in placentas of high BMI women (0.110 ± 0.027 pmol/mg x 10sec) as compared to the control group (0.122 ± 0.022 pmol/mg x 10sec). Moreover, MVM protein expression of the System L isoform LAT1 was not changed by maternal BMI.

CONCLUSION: These results suggest that the activity of placental amino acid transporter Systems A and L is not affected by overweight/obesity in Hispanic women. This is in contrast to our previous report of up-regulation of placental System A activity in high BMI Swedish women (Jansson N et al 2010, Reprod Sci 17: 260A), suggesting interesting ethnic differences in the placental response to maternal obesity. The larger placental size in the high BMI group is consistent with an increased nutrient transport capacity, which could contribute to the higher birth weights observed in this group. Further studies will establish whether mTORC1 activation results in up-regulation of other nutrient transporters in the placenta of obese Hispanic women.

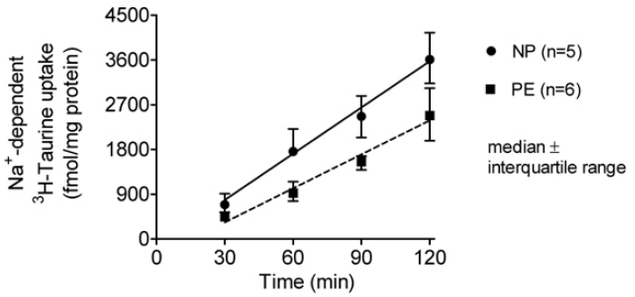
S-142

Reduced Placental Taurine Transport in Pre-Eclampsia. Chloe R Hirst, Susan L Greenwood, Michelle Desforges. *Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Background: Pre-eclampsia (PE) is associated with abnormal renewal of placental syncytiotrophoblast by cell turnover. The amino acid taurine is an intracellular osmolyte that regulates proliferation, differentiation and apoptosis in many tissues. We showed differentiation of trophoblast cells *in vitro* is compromised and apoptotic susceptibility is increased following siRNA-mediated taurine transporter (TauT) knockdown (Parsons *et al* 2009. Placenta 30: A.83, Desforges *et al* 2010. Reprod Sci 3:317A). Here we test the hypothesis that syncytiotrophoblast TauT expression and activity is reduced, and intracellular taurine is lower, in PE compared to normal pregnancy (NP).

Methods: Placentas were collected from NP and PE (blood pressure >140/90mmHg after 20wks gestation in previously normotensive women plus proteinuria >300 mg/L in a 24hr collection). TauT protein expression in membrane-enriched placental homogenates was assessed by Western blotting. Nitrocellulose membranes were re-probed for β-actin to normalise TauT expression. TauT activity was measured as initial rate of Na^+-dependent uptake of ^{3}H-taurine into placental villous fragments and intracellular taurine was estimated by measuring ^{3}H-taurine accumulation by fragments at steady state (20hr).

Results: TauT protein was observed at 70 and 50kDa consistent with previous Western blot analysis of human placenta (Roos *et al*. 2004). There was no difference in the density of immunoreactive signals between NP and PE (n=6, Mann Whitney). TauT activity was significantly reduced in PE compared to NP (figure: $p<0.05$, linear regression). Preliminary data indicated that this resulted in lower intracellular taurine in placentas from PE (n=3) compared to NP (n=5) (^{3}H-taurine accumulation 1.5-1.8 vs. 1.5-3.2 mmol/mg protein respectively).



Conclusion: Placental TauT activity, but not expression, is lower in PE than normal pregnancy. Similar observations have been reported previously in placentas from fetal growth restriction (Norberg *et al*. 1998, Roos *et al*. 2004). Reduced TauT activity could contribute to increased apoptosis and aberrant trophoblast cell turnover in these pregnancy complications.

S-143

Folate Depletion in a Maternal Low Protein Diet Changes Placental Gene Expression. Melissa Hum,[1] Marloes Dekker Nitert,[1] Ryan J Wood-Bradley,[2] James A Armitage,[2] Murray D Mitchell,[1] Gregory E Rice.[1] *[1]UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia; [2]Anatomy and Developmental Biology, Monash University, Melbourne, Victoria, Australia.*

Introduction: Maternal low protein diet induces low birth weight of rat offspring and predisposes the offspring to increased risk of developing metabolic disease later in life. These changes are partially transmitted by changes in DNA methylation. Folate is a methyl donor and adequate levels are required for optimal DNA methylation to occur.

Objective: To compare genome-wide gene expression levels in placenta in rats fed a low protein diet depleted of or supplemented with folate.

Methods: Female Sprague Dawley rats were fed an isocaloric low protein diet (8.4% protein vs 20% protein in control diet) supplemented with 200 mg/kg folate (n=4) or depleted of (<0.05 mg/kg folate) folate (n=4) for 3 weeks, mated with male Sprague Dawley rats on a normal diet and kept on the assigned diet throughout pregnancy. Rats were sacrificed at E17.25 days gestation and placentae were taken. Total RNA was isolated and hybridized to Illumina rat gene expression arrays (Sentrix RatRef-12 Expression BeadChip). Data was normalized with a VST transformation followed by aRSN normalization and analyzed with the SAM (Significance Analysis of Microarrays) software tool. False discovery rate was used to adjust for multiple testing.

Results: Folate depletion did not affect placental weight or offspring weight at E17.25 days gestation. Folate depletion significantly decreased the placental expression of 9 genes, 4 of which have a known function including the NFkB repressing factor (*Nkrf*), the glucose carrier (*Slc35b1*), the ADP-ribosylation factor 1 (*Arf1*) and the Pseudouridylate synthase 7 homolog (*Pus7*). There were no statistically significant upregulated genes.

Discussion: Folate depletion decreases the expression of genes involved in the regulation of inflammation (Nkrf), glucose transport (Slc35b1) and protein trafficking (Arf1) in placentae of rats fed a low protein diet. Further analysis of the data generated by this project will be performed and will include confirmation of the microarray results by Q-PCR and immunoblot and DNA methylation analysis of relevant promoters.

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Enhanced Placental Glucose Transport Mechanisms May Contribute to the Correction of Placental Insufficiency by Direct Placental Adenoviral-Mediated hIGF-1 Transfer in a Murine Model. Helen Jones, Chuck Klanke, Stephanie Lang, Mounira Habli. *Center for Molecular Fetal Therapy, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.*

Previous work in our laboratory demonstrated that over-expression of human insulin-like growth factor -1 (hIGF-1) in the placenta corrects fetal weight deficits in a mouse model of fetal growth restriction with associated changes in placental GLUT1 relocalization. However, two isoforms of GLUT9 were recently identified in the mouse liver and human placenta and shown to be differentially regulated in diabetes and pathological pregnancies (1, 2). It is unknown if these novel transporters are expressed in mouse placenta, altered in placental insufficiency (PI) or regulated by IGF-1. To investigate a potential role of GLUT9 in PI and intra-placental hIGF-1 over-expression we examined expression and localization in a mouse model of IUGR.

Methods: At gestational day 18, animals were divided into three groups; sham-operated controls, uterine artery branch ligation (UABL), UABL + Ad-hIGF-1 (10^8 PFU). At gestational day 20, pups and placentas were harvested by C-section. The RNA and protein expression and localization of glucose transporters GLUT9a and GLUT9b were analyzed by RT-PCR and immunohistochemistry.
Results: Total GLUT9 RNA expression was unaltered in any of the 3 experimental groups. Protein expression was minimal for both isoforms of GLUT9 in sham and UABL placentas, however positive staining for GLUT9a and b was seen in Ad-hIGF-1 treated placentas in the labyrinthine zone. Neither isoform demonstrated preferential membrane localization in the murine syncytiotrophoblast.
Conclusion: GLUT9 isoform localization does not reflect the distinct pattern seen in the human placenta. Expression levels of both GLUT9 isoforms are low in the mouse placenta and uterine artery branch ligation does not alter GLUT9a or b expression, however hIGF-1 over-expression enhances both GLUT9a and b protein expression. This enhanced protein expression appears to be independent of an increase in RNA expression suggesting the involvement of post-transcriptional regulatory pathways. Upregulation of GLUT9 may represent one mechanism involved in Ad-hIGF-1 correction of placental insufficiency and associated fetal growth restriction.
1. Keembiyehetty C., et al. 2006. Molecular Endocrinology 20:686-697
2. Bibee K. P., et al. 2011. Reproductive Sciences 18:20-27

S-145

Does Antithrombotic Treatment Have a Trophic Effect on Neonatal Birthweights in Patients at Risk for Adverse Pregnancy Outcomes? Joel D Kamda,[1] John C Pezzullo,[3] Alessandro Ghidini,[2] Sarah H Poggi.[1,2] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, INOVA Alexandria Hospital, Alexandria, VA, USA; [3]Medicine, Georgetown University Hospital, Washington, DC, USA.*
OBJECTIVE: To determine if low molecular weight heparin (LMWH) treatment is associated with enhanced birthweight (BW) centiles in patients at risk for recurrence of placenta-related adverse pregnancy outcomes (APO)
STUDY DESIGN: This is a retrospective 5 year cohort study of pregnant women (N=128) with history of APO including severe preeclampsia (n=28), placental abruption (n=4), fetal loss >20 wks (n=47), recurrent spontaneous abortions (n=25). LMWH prophylaxis (Lovenox 40 mg daily) was left to the discretion of the managing physician. Excluded were cases requiring LMWH prophylaxis for venous thromboembolism. Neonatal BW centiles (adjusted for gestational age at delivery and gender) were related to obstetric variables using linear regression analysis.
RESULTS: The overall recurrence of APO was 21% (27/128). Univariate anlaysis revealed no significant difference in BW centile between the LMWH treated (n=40) vs untreated group (53 ± 28 vs 48 ± 28, P=0.4). Linear regression analysis identified history of preeclampsia (p=0.012, coefficient -15) and maternal age (p=0.05, coefficient +0.87) as the only significant predictors of BW centile.
CONCLUSION: In women at high risk for fetal growth restriction due to history of placenta-related APO, use of LMWH is not associated with an increase in neonatal BW centile. History of preeclampsia is strongly associated with lower BW centiles and the association is not mitigated by LMWH.

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Down Regulation of Placental Amino Acid and Glucose Transporters in Response to Maternal Nutrient Restriction (MNR) in the Baboon. Jovita V Kavitha,[1] Peter W Nathanielsz,[1] Thomas J McDonald,[1] Guoyao Wu,[2] Theresa L Powell,[1] Thomas Jansson.[1] *[1]Center for Pregnancy and Newborn Research, Dept of OB/GYN, Univ of Texas Health Science Center, San Antonio, TX; [2]Dept of Animal Science, Texas A&M University, College Station, TX.*
Introduction: The mechanisms underlying reduced fetal growth in response to MNR remains to be fully established. We hypothesized that MNR in the baboon inhibits placental mTOR signaling, down regulates the expression of placental amino acid and glucose transporters and decreases fetal circulating levels of amino acids. **Methods:** Pregnant baboons were fed either control diet (C; ad lib, n=8) or a MNR diet (70% of diet consumed by controls, n=8) from Gestational Day (GD) 30. At GD 165 the placenta and umbilical blood were collected at cesarean section. Western blot was used to determine the protein expression of total and phosphorylated 4E-BP1 and S6 ribosomal protein in placental homogenates and of glucose transporter 1 (GLUT1), sodium coupled neutral amino acid transporter (SNAT) and large neutral amino acid transporter (LAT) isoforms in syncytiotrophoblast microvillous membranes (MVM). Fetal serum amino acids were quantified by HPLC. **Results:** Fetal weights (C: 774.1 ± 43.2 g, MNR: 702.6 ± 37.8 g) and placental weights (C: 185.1 ± 11.1 g, MNR: 157.2 ± 36.7 g) were decreased in the MNR group, however this difference did not reach statistical significance. Expression of phosphorylated 4E-BP1 (Thr37/46, -30%, $p<0.05$) and S6 ribosomal protein (Ser235/236, -35%, $p<0.05$), two functional read outs of mTOR activity, was reduced in response to MNR. In MNR placentas, MVM GLUT1 ($p<0.05$), SNAT2 ($p<0.05$) and LAT 2 ($p<0.01$) expression, but not the expression of SNAT1 and 4, was reduced as compared to control. Fetal serum concentrations of certain essential amino acids were lower in the MNR group (Table 1). **Conclusion:** MNR in baboon pregnancy inhibits placental mTOR signaling and down regulates placental GLUT1 and System A and L amino acid transporter expression. We speculate that these changes contribute to decreased fetal nutrient availability and reduced fetal growth.

Table 1. Fetal serum amino acid concentrations (μM) at GD 165

	Control (n=21)	MNR (n=6)	Difference (%)	P-value
Tyrosine	63.3 ± 4.0	42.0 ± 2.3	-34	<0.01
Taurine	206.3 ± 12.1	140.6 ± 17.5	-32	<0.05
Methionine	45.1 ± 1.8	38.4 ± 2.4	-15	0.06
Leucine	108.3 ± 5.6	73.6 ± 2.2	-32	<0.05
Phenylalanine	98.6 ± 6.0	63.2 ± 3.6	-36	<0.01

Means ± SEM, unpaired t-test. Amino acids in bold are essential.

S-147

Pericytes in Human Placental Terminal Villi Are Members of the Blood-Placenta Barrier and Regulate Capillary Diameter; Implication for IUGR Pathophysiology. Umit A Kayisli, Emre Vatanadaslar, Nehir Ocak, Harvey Kliman, Frederick Schatz, Charles J Lockwood. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Background: As pregnancy progresses, the blood-placenta barrier in mature terminal villi thins. At term, it is comprised of a syncytial layer, a common basal membrane of the syncytium merged with fetal capillary endothelium, and a fetal capillary endothelium. Surrounding capillaries, pericytes provide vascular support by forming a monolayer of smooth muscle like-cells with high contractility regulating endothelial differentiation, capillary permeability, plasticity and blood flow.
Objectives: Determine time of differentiation and localization of pericytes in placentas. Compare the diameters of terminal villi capillaries in normal and intrauterine growth restricted (IUGR)-placentas.
Methods: Paraffin sections (5μm) obtained from 1st (n=4) and 2nd trimester (n=4) elective terminations and 3rd trimester (n=4) normal deliveries were immunostained for pericyte markers α-smooth muscle actin (α-SMA) and CD90. Capillary diameters were measured in 10 normal (n=541vessels) and 10 IUGR (n=481 vessels) gestational age matched placentas. After H&E staining, diameters were determined with microscopic imaging analysis.
Results: Pericyte-like cells (PLC) surrounding capillaries in placental villi from 1st trimester displayed strong immunoreactivity for CD90 but were negative for α-SMA. Similarly, PLC from 2nd trimester also exhibited immunoreactivity for CD90, but weak to moderate immunoreactivity for α-SMA. In 3rd trimester, PLC were in much closer proximity to capillaries and located between the syncytium and capillary where the blood-placenta barrier established. These PLC displayed strong immunoreactivity for α-SMA, but weak CD90 immunoreactivity. In normal placentas, mean capillary diameters were 4.7±0.07 μM and significantly greater than that in IUGR placentas (4.3±0.06μM); $p<0.01$.
Conclusions: We reveal for the first time that pericytes begin to differentiate in the 2nd trimester and this differentiation is completed during the 3rd trimester. Because of their location between the syncytium and endothelium in the terminal villi, these cells deserve recognition as members of the blood-placenta barrier. Smaller capillary diameters in terminal villi of IUGR placentas strongly support that pericytes may be involved in the physiopathologic regulation of placental microcirculation.

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Expression of Podocyte Related Protein in the Human Placenta. Jung Hwa Park,[1] Ja Young Kwon,[1] Bo Hyun Yoon,[1] Young Han Kim,[1] Ga Hyun Son,[1] Hyung Min Choi,[2] Yong Won Park.[1] *[1]Department of Obstetrics and Gynecology, Yonsei University College of Medicine, Yonsei University Health System, Seoul, Republic of Korea; [2]Department of Obstetrics and Gynecology, Inje University, Ilsan Paik Hospital, Ilsan, Republic of Korea.*
Objective: Podocyte related proteins are cell membrane proteins expressed in the slit diaphragm of the renal glomeruli serving as a tight barrier separating vascular and urinary spaces. One of the roles the placental system plays is a

barrier between fetal and maternal compartments restricting over exchange. Thus, the purpose of the study is to search for their presence in the human placenta.
Methods: A normal healthy pregnant women who underwent cesarean section at Yonsei University Health System from January to May 2011 were the candidates for this study. Four parts including chorion, amnion, villus were obtained from placental tissues. Real-time PCR, western blotting, and immunohistochemical staining were performed for nephrin, NEPH1, NEPH2 and synaptopodin.
Results: A total of 10 women were consented to the study. The mean age of the group was 34 years at 38 gestational weeks average. Nephrin, NEPH1, NEPH2, and synaptopodin were all expressed in villus, amnionic, and chorionic membranes.
Conclusion:This is the first to report the presence of podocyte related protein in the human placenta. Although its role remains unclear, it may play a crucial role as a maternal-fetal barrier.

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Placental Expression of Fatty Acid Transport Protein 4 in Lean and Overweight Women. Susanne Lager, Thomas Jansson, Theresa L Powell. *Center for Pregnancy and Newborn Research, Dept. of OB/GYN, University of Texas Health Science Center, San Antonio, TX, USA.*
Background An adequate supply of fatty acids is important for normal fetal development and growth. Long-chain polyunsaturated fatty acids (LCPUFAs), such as docosahexaenoic acid, are critical for proper neurological and visual development. The fetus depends on maternal supply of LCPUFAs, since the ability to convert essential fatty acids into LCPUFAs is limited in the fetus and placenta. Fatty acid transport protein 4 (FATP4) facilitates uptake and/or directly transports long-chain fatty acids. Women with elevated body mass index (BMI) are more likely to deliver a large for gestational age infant with increased fat deposition. We tested the hypothesis that FATP4 expression is increased in placenta from women with high BMI.
Methods Term placenta were collected from lean (mean BMI 21.5±0.7 kg/m^2, n=10) and overweight women (mean BMI 27.0±0.8 kg/m^2, n=5) based on pre-pregnancy BMI. Placental basal plasma membranes (BM) and microvillous plasma membranes (MVM) were isolated. Protein expression of FATP4 was determined by western blot. Differences between the groups were evaluated statistically by Mann-Whitney test.
Results Birth weights did not differ significantly between groups (lean: 3345±79 *vs.* obese: 3601±205 g). The ponderal index was lower in the infants born to lean women (2.48±0.06 *vs.* 2.80±0.11 g/cm^3 x100; p<0.05). There was a 49% reduction in MVM expression of FATP4 (p<0.05) with maternal overweight. In contrast, BM FATP4 expression increased by 45% in the overweight group, however this difference did not reach statistical significance (p=0.08). The opposing changes in the two membrane surfaces resulted in a 68% decrease in the FATP4 MVM/BM ratio (p<0.01) with high BMI.
Conclusion The localization of FATP4 protein to both plasma membranes of the syncytiotrophoblast has not been previously reported. These preliminary data suggest that the placenta in overweight women has a reduced capacity for uptake of long-chain fatty acids from the maternal circulation, whereas the capacity to transfer these fatty acids into the fetal circulation across the BM may be enhanced. Further studies are needed to determine the effect these changes on net transfer and on fetal fatty acid accumulation.

S-150

Maternal Smoking Alters Placental 11βHsd2 mRNA and Serum Corticosterone Levels in a Gender-Specific Manner in the Rat Pup. Gwen Latendresse,[1] Merica Hale,[2] Chengshe Jiang,[2] Melanie Fitzhugh,[2] Christopher Calloway,[2] Camille Fung,[2] Dan Malleski,[2] Robert Lane,[2] Lisa Joss-Moore.[2] *[1]College of Nursing, University of Utah, Salt Lake City, UT, USA; [2]Pediatrics, Division of Neonatology, University of Utah, Salt Lake City, UT, USA.*
BACKGROUND: Maternal tobacco smoke (MTS) exposure increases the risk of intrauterine growth restriction (IUGR). IUGR is a significant cause of newborn morbidity and mortality, with males more severely affected. Altered metabolism of glucocorticoids (GC) in the placenta contributes to IUGR. Placental GC metabolism is regulated by the GC receptor (GR) as well as hydroxysteroid dehydrogenase type 2 (11βHsd2), which metabolizes GC into an inactive form, and 11βHsd1 which converts inactive GC back to active GC. Despite these connections, It is unknown if MTS differentially alters placental GC metabolism in the male vs. female rat pup.
OBJECTIVE: We hypothesize that MTS alters mRNA levels of 11β-Hsd1, 11β-Hsd2, and GR in the rat placenta, as well as pup serum corticosterone levels.
METHODS: Pregnant dams were exposed to tobacco smoke from e11 to term (e21). Placentae and serum were collected from MTS pups and controls delivered via cesarean. Total placental mRNA transcript levels were quantified using real-time PCR. Pup serum cortiscosterone concentrations were measured using ELISA. * p<0.05**p<0.01
RESULTS: In the female pup placenta, MTS did not alter 11β-Hsd1, 11β-Hsd2, and GR mRNA levels. However, serum corticosterone levels were decreased by 48%* relative to sex-matched controls. In the male pup, MTS increased placental 11b2 mRNA levels by 192%.** Serum corticosterone levels were also increased by 185% in the male pups, however the increase did not reach statistical significance (p = .11).
DISCUSSION: We showed that MTS affects 11β-Hsd2 mRNA levels in the male, but not in the female placenta. Furthermore, MTS differentially alters serum corticosterone levels in male and female pups. We speculate that 11β-Hsd2 protein levels and activity is increased in male rat pup placenta in accordance with increased mRNA. We further speculate that altered GC metabolism in the male pup placenta could be a contributing factor to poorer outlook for male IUGR offspring of MTS exposed dams.

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Ferroportin Expression in Human IUGR Placentas. Chiara Mando,[1,2] Maria Antonella Marino,[1,2] Giovanni Francesco Russo,[1,2] Martina Ilaria Mazzocco,[1,2] Irene Cetin. *[1]Clinical Sciences L.Sacco, University of Milan, Milan, Italy; [2]Center for Fetal Research Giorgio Pardi, University of Milan, Milan, Italy.*
Background
Iron (Fe) deficiency in pregnancy is associated with low birth weight and premature delivery.
We previously demonstrated a significant decrease of the Fe cell-importer Transferrin Receptor (TfR1) in human Intrauterine Growth Restricted (IUGR) vs normal (N) placentas.
Ferroportin (FPN) is highly expressed in the basolateral membrane of the syncytiotrophoblast (STB), exporting Fe from the STB to the fetal circulation. Its mutations have been described in a form of haemochromatosis. FPN is regulated by several factors, particularly hypoxia and acute inflammation, typical features of IUGR placentas. Here, we hypothesized that the previously reported TfR1 downregulation in IUGR placentas could be due to Fe intracellular accumulation, and we measured FPN expression in human IUGR vs N placentas.
Methods
Placentas were sampled at the time of elective cesarean section; villi were selected, washed and immediately frozen for following analysis. IUGR was defined by a reduction of more than 40 centiles in the growth of the abdominal circumference measured by ultrasound in utero and birth weight <10th percentile. Three severity groups were identified depending on the umbilical artery pulsatility index and fetal heart rate. FPN mRNA was quantified in 41 IUGR and 50 N placentas by Real Time PCR. Enzyme-Linked ImmunoSorbent Assay (Elisa) protocol for FPN protein expression was set up, and 7 IUGR and 7 N placentas were analyzed.
Results
FPN mRNA expression was not statistically different in IUGR placentas, independently from degree of severity. FPN proteins showed a non significant trend towards a lower expression in IUGR placentas compared to N.
Discussion
This is the first investigation of FPN expression in human IUGR placentas. Our preliminary results show no differences in IUGR and N FPN mRNA and protein placental levels. This suggests that the Fe reaching IUGR fetuses could be decreased compared to normal pregnancies, as a consequence of TfR1 downregulation in the microvillous membranes. Since FPN is known to be post-transcriptionally finely regulated, we aim at enlarging our Elisa analysis case study in order to confirm our data, and then measuring Fe levels in the cord blood to verify our hypothesis.
(Supported by Fondazione Giorgio Pardi)

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Increased Perinatal Loss in D6 Deficient Mice. Pek Joo Teoh,[1] Graham J Burton,[2] Rob J Nibbs,[3] Scott M Nelson.[1] *[1]Reproductive and Maternal Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Centre for Trophoblast Research, University of Cambridge, Cambridge, United Kingdom; [3]Infection, Inflammation and Immunity, University of Glasgow, Glasgow, United Kingdom.*
Introduction: D6, a regulator of chemokine abundance, is highly expressed by the placenta and has been shown to protect against fetal death induced by bacterial endotoxin or anti-phospholipid antibodies from patients with

anti-phospholipid syndrome, and aids fetal survival after embryo transfer into allogeneic recipients. To investigate this further we assessed the impact of d6 deletion on perinatal outcomes of naturally conceived syngeneic fetuses in unchallenged DBA/1 mice, postnatal growth, d14 and d18 placental stereology. *Results:* The lack of D6 caused a highly significant increase in the frequency of stillbirth (10.3% of wild-type pups (n=474) vs 21.3% of D6 deficient pups (n=418); $p<0.0001$ ($\chi 2$ test)) and neonatal death in first 3 wks after birth (16.7% of wild-type pups (n=400) vs 37.0% of D6 deficient pups (n=322); $p<0.0001$ ($\chi 2$ test)), leading to a substantial reduction in mean number of pups successfully weaned per litter (4.2+/-0.3 wild-type pups/litter (n=80 litters) vs 2.7+/-0.3 D6 deficient pups/litter (n=75 litters); p=0.0018 (Mann Whitney)). Assessment of postnatal weight over the first 20 days of life (weaning), did not demonstrate a significant difference. To examine whether the phenotype was related to abnormal placental development, time dated mice were assessed on d14 and d18. Fetal weight but not placental weight was significantly reduced in the d6 deficient mice on day 14 (n=44 WT, n=24 D6 deficient; p=0.003)). Detailed placental stereology of 8 mice per group demonstrated that on d14 the Labyrinthine Zone volume % (LZ) was significantly reduced in the d6 deficient mice (p=0.03), with a concomitant increase in the Junctional Zone volume % (JZ) (p=0.03). LZ was positively associated with fetal weight (r=0.52, p=0.04), with the JZ negatively associated (r=-0.5, $p<0.05$). The decidual basalis and chorionic plate volumes were similar in both groups. These differences were not observed on d18.
Conclusion: The D6 receptor is critical for normal LZ development and fetal growth, however, late compensatory mechanisms exist to ensure normal fetal weight at term. This initial abnormal placental development may underlie the observed increase in stillbirth and neonatal deaths.

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Placental Myostatin Expression throughout Pregnancy in the Spiny Mouse. Hassendrini N Peiris,[1] Bree O'Connell,[2] Hayley Dickinson,[2] David W Walker,[2] Murray D Mitchell.[1] *[1]UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia; [2]The Ritchie Centre, Monash Institute of Medical Research, Melbourne, Victoria, Australia.*
Background: Inadequate placental development leads to pregnancy disorders and can adversely affect the postnatal life of offspring. Myostatin is a negative regulator of muscle development affecting proliferation and differentiation. Within the placenta, myostatin is known to alter glucose uptake, however, the variation of myostatin expression across gestation is unknown. Spiny mice (*Acomys cahirinus*) are a species of rodent with a long period of gestation (39-days) and precocial offspring (completed organogenesis at birth). These unique features of the spiny mouse allow for easier translation of findings to human development.
Objective: To assess placental myostatin protein expression throughout pregnancy in the spiny mouse.
Methods: Protein was extracted and concentration determined. Western blots were undertaken for the detection of the myostatin precursor (~52kDa) and the biologically active myostatin dimer (~28kDa). Relative optical densities (OD) were determined using a densitometer utilising Quantity One software. Spiny mouse placentae were removed, weighed and snap frozen from gestational age (GA) 15 to 38 days.
Results: The placental expression of myostatin throughout gestation peaks during mid-gestation (GA20 to 25) and falls toward term (GA30 to 38). Significant differences ($p<0.05$; $p<0.001$) in myostatin precursor concentrations were identified when comparing the placentae from GA15 to 19 (0.017±0.002) and GA30 to 38 (0.013±0.001) with GA20 to 25 (0.026±0.003). Similarly significant differences ($p<0.001$) of myostatin dimer concentrations were identified when comparing the placentae from GA15 to 19 (0.014±0.001) and GA30 to 38 (0.013±0.001) with GA20 to 29 (0.028±0.002).
Conclusions: The peak expression of myostatin, GA20 to 25, coincides with the development of the labyrinth region of the spiny mouse placenta. The labyrinth is comparable to the fetal portion of the human placenta involved in the exchange between mother and fetus. Hence determination of placental myostatin across gestation may permit a better understanding of placental development and could lead to the development of therapeutic interventions and diagnostic tools to treat conditions such as placental insufficiency.

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Folate Deficiency Inhibits Mammalian Target of Rapamycin (mTOR) Signaling in Cultured Primary Human Trophoblast Cells. Fredrick J Rosario, Theresa L Powell, Thomas Jansson. *Center for Pregnancy and Newborn Research, Dept OB/GYN, University of Texas Health Science Center, San Antonio, TX, USA.*
Introduction: Poor maternal folate status has been associated with intrauterine growth restriction (IUGR) and folate supplementation in women with folate deficiency increases birth weight. However the mechanisms linking maternal folate levels to fetal growth remain to be fully established. Mammalian target of rapamycin (mTOR) is a protein kinase that has been proposed to function as a placental nutrient sensor. Placental mTOR is a positive regulator of placental nutrient transport, thereby affecting fetal growth, and has been reported to be inhibited in IUGR. We hypothesized that folate deficiency inhibits placental mTOR signaling. **Methods:** Human primary cytotrophoblast cells were isolated from normal term placentas and cultured in media with and without folate for 90 hours to allow for differentiation. Subsequently, cell lysates were obtained and the expression of p53, syncytin, caspase-3, total and phosphorylated S6 ribosomal protein (Ser-235/236), and 4E-BP1 (Thr-37/46 and Thr-70) was determined using Western blot. **Results:** Folate deficiency for 90 hours did not affect syncytialization or apoptosis as determined by the expression of p53, syncytin and caspase-3. Folate deficiency did not significantly alter the expression of total S6 ribosomal protein, total 4E-BP1 or phospho-4E-BP1 (Thr-70). Expression of phosphorylated 4E-BP1 (Thr-37/46) was reduced by 59 ± 5 % (RM ANOVA, P=0.03, n=6) and phosphorylated S6 ribosomal protein (Ser-235/236) was reduced by 62 ± 12 % (RM ANOVA, P=0.02, n=6) in response to folate deficiency. **Conclusion:** Our data suggest that folate deficiency inhibits mTOR signaling in cultured primary human trophoblast cells. This is the first report, in any cell type, to show that folate is an upstream regulator of mTOR. We speculate that mTOR regulation of placental nutrient transport may be one mechanism linking maternal folate status to fetal growth.

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Investigation for the Effect of the Environmental Contaminants Exposure on the Placental Amino Acid Transport Activity. Eiji Shibata,[1,2] Toshihiro Kawamoto,[2] Yukiyo Aiko,[1,2] David J Askew,[2] Rei Suga,[2] Mai Myoga,[1] Toru Hachisuga.[1] *[1]Department of Obstetrics and Gynecology, University of Occupational and Environmental Health; [2]Maternal and Child Environmental Health Center, University of Occupational and Environmental Health.*
(Background)
Recent studies indicate that maternal exposure to environmental contaminants may decrease infant birth-weight. However, underlying mechanisms of fetal growth restriction caused by environmental contaminants is not understood. The aim of this study is to investigate in vivo levels of environmental contaminants in the mother and infant, and to clarify how those chemicals decreases the infant birth-weight.
(Subjects and Methods)
We measured in vivo levels of heavy metals including Lead, Arsenic, Cadmium, Mercury, and various pesticides in maternal and cord blood, and maternal urine of the time of delivery. Furthermore, we exposed villous fragments, and BeWo cell to Arsenic, Cadmium, Mercury, plastic resin; toluene diisocyanate, aldehyde analog; glutaraldehyde, acetaldehyde, and formaldehyde to mesure the placental system A transport activity, and system A and L amino acid transporter protein expression.
(Result)
In vivo experiments (n=9); All of environmental contaminants were under the Japanese environmental criteria. Total Mercury level was higher in cord blood than in maternal blood. Cadmium was detected in maternal specimens but not in the cord blood. In vitro experiments (n=6); Methyl-Mercury (0.5μM), Arsenic (5μM), Cadmium (20μM) were all found to decreased system A amino acid transport activity by 30 to 40% ($P<0.001$). Toluene diisocyanate and glutaraldehyde decreased system A amino acid transport activity significantly in a dose-dependent manner (1, 10, 100μM: $P<0.001$), but acetaldehyde (2.3, 23, 230μM) and formaldehyde (3.3, 33, 330μM) did not. Toluene diisocyanate and glutaraldehyde did not change expression of System A or L amino acid transporter protein in the cultured villous fragments or the BeWo cell line. All environmental contaminants tested did not increase the lactase dehydrogenase release into the culture media.
(Conclusion)
We suggest that the high concentration of Methyl-Mercury, Arsenic, and Cadmium decrease system A amino acid transport activity. Toluene diisocyanate

and glutaraldehyde are thought to be environmental contaminants which may cause fetal growth restriction by decreasing placental system A amino acid transport activity.

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Increase Phosphorylation of Eukaryotic Initiation Factor 2 Alpha and 4E Binding Protein 1 Expressions in High Altitude Placentas: The Role of Protein Synthesis Inhibition in Small for Gestational Age Babies. Hong wa Yung,[1] Mathew J Cox,[1] Martha C Tissot van Patot,[2] Graham J Burton.[1] *[1]Centre for Trophoblast Research, University of Cambridge, Cambridge, United Kingdom; [2]Denver Health Sciences Center, University of Colorado, Aurora, CO, USA.*

Pregnancy at high altitude is associated with SGA babies, with a reduction of 100g in birth weight per 1000m of altitude. Although hypobaric hypoxia is the obvious key factor, the molecular mechanisms involved are unclear. AKT-mTOR signalling regulates protein translation, growth and proliferation. In human pathological IUGR, loss of AKT-mTOR signalling and protein synthesis inhibition has been demonstrated to be one of the possible underlying mechanisms. Therefore, we tested for evidence of these changes in high-altitude placentas.

METHODS: Snap-frozen samples from placentas delivered by elective caesarean section from normotensive women at sea level and 3100m (Leadville, Colorado) were used to analyse the AKT-mTOR signalling. Trophoblast-like cells and primary placental fibroblasts were cultured under 1% and 10% O2 to test activation of the same pathways by hypoxia.

RESULTS: High-altitude placentas displayed strong immunostaining for lipid peroxidation (4-HNE), and high phosphorylation of p38 MAPK and heat shock protein 27 (HSP27) compared to sea-level controls. There was increased phosphorylation of eIF2α and reduced AKT signalling. mTOR complex 1 signalling, which regulates protein synthesis, was compromised as indicated by reduction of phosphorylation of TSC2 and 4E binding protein 1 (4E-BP1). Total 4E-BP1 protein was also increased. Increased P-eIF2α, reduced AKT signalling and reduced proliferation were observed in both trophoblast-like cells and placental fibroblasts cultured under 1% O2.

CONCLUSION: Increased 4-HNE immunostaining, P-p38 MAPK and P-HSP27 indicate high-altitude placentas suffer from oxidative stress. Down-regulation of AKT and mTOR signalling, with high level of 4E-BP1 total protein and increased P-eIF2α provides evidence of protein synthesis inhibition, and can be mimicked in in vitro cell culture through hypoxia. Reduction of protein synthesis will limit growth of the placenta, which in turn restricts fetal growth.

Supported by Wellcome Trust.

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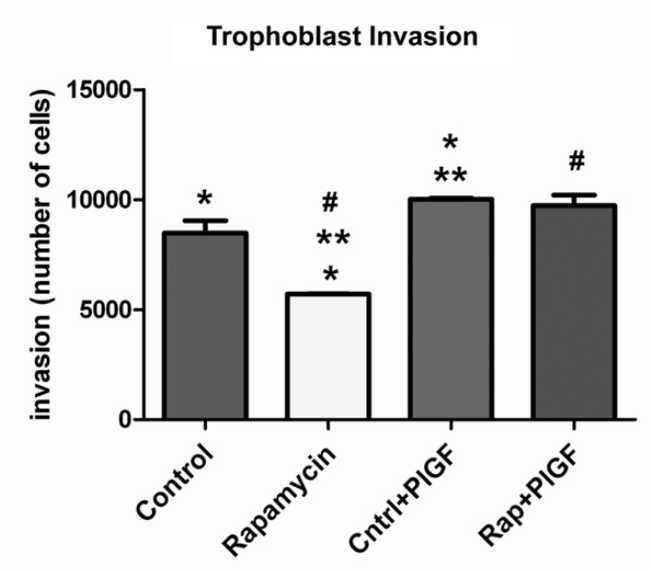
Placenta Growth Factor (PGF) Induces Invasion and Activates p70 during Rapamycin Treatment in Trophoblast Cells. Amanda Graham,[1] Donald S Torry,[2] Juan A Arroyo.[1] *[1]Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS, USA; [2]Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL, USA.*

Objective: Trophoblast are specialized epithelial cells that are critical for successful pregnancy. Aberrant trophoblast invasion has been associated with obstetric pathologies including human intrauterine growth restriction (IUGR) and preeclampsia (PE). IUGR is also associated with decrease in PGF production and downregulated mTOR pathway signaling. Our objective was to determine PGF-mediated regulation of cell invasion in trophoblast cells with reduced mTOR signaling.

Methods: First trimester SW71 trophoblast cells were subjected to matrigel invasion assays with the following conditions: 10% FBS, 10% FBS with Rapamycin and 10% FBS with Rapamycin and PGF. Cells were cultured and western blots performed on cell lysates with antibodies against phosphor- and total mTOR, p70, 4EBP1, ERK and AKT. Actin westerns on the same blots were used to account for inter-lane loading variation.

Results: Compared to controls, trophoblast cells showed: 1) a 33% decrease in invasion ($p<0.05$) following Rapamycin treatment, 2) no significant differences in invasion following Rapamycin and PGF treatment, 3) a 1.4-fold decrease ($p< 0.005$) in mTOR activation with Rapamycin, and 4) an increased activation of p70 (2.3-fold), ERK (3.5-fold) and AKT (1.7-fold) with Rapamacyn and PGF treatment.

Conclusions: First trimester trophoblast invasion is functionally decreased when activation of mTOR is prevented and this decrease is recovered with the addition of PGF. Mechanistically, this recovery involves the activation of the p70 and AKT pathways independent of mTOR. These studies may be of clinical significance in conditions of IUGR and PE where trophoblast invasion and PGF levels are reduced.



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De-Methylation of Slug in 1st Trimester Extravillous Trophoblast Cell Line HTR8/SVneo Epigenetically Describes Trophoblast Epithelial-Mesenchymal Transition. Ying Chen, Kai Wang, Richard Leach. *Ob/G. & Reproductive Biology, Michigan State University, Grand Rapids, MI, USA.*

Background: During trophoblast invasion, cytotrophoblast (CT) turn into invasive extravillous trophoblast (EVT), it is an epithelial-mesenchymal transition (EMT). Although epigenetic mechanism has been well studied in cancer EMT, little is known about the trophoblast EMT. Stem cell mesenchymal differentiation is a typical EMT as well. The comparison of trophoblast EMT and stem cell mesenchymal differentiation EMT could point to the specificity of trophoblast EMT. Snail and Slug are EMT master genes; they repress multiple epithelium genes and maintain mesenchymal morphology. In this study, we investigated the role of DNA methylation on the regulation of Snail and Slug genes in trophoblast cell lines. Further, we used DNA methylation status and mRNA level of Snail, Slug and MMP3 to describe the EMT process of trophoblast cell compared with the EMT of stem cell mesenchymal differentiation.

Methods and Results: Firstly, when we applied 5'AZA treatment on BeWo (CT-like) and HTR8/SVneo cells (EVT-like), Snail and Slug genes increased expression detected by real-time PCR after 3 days treatment. Furthermore, we cloned both gene promoters into pGL3-Basic vector upstream of luciferase, after in vitro methylation; these promoter vectors were used to transfect IMR90 cells. We found luciferase levels of methylated vectors were much less than un-methylated vectors. These results demonstrated that transcription of Snail and Slug genes were regulated by DNA methylation in trophoblast cells. Next, we compared DNA methylation and mRNA levels of Snail, Slug and MMP3 between BeWo with HTR8/SVneo trophoblast cells, and between iPS cells and IMR90 cells to describe EMTs in trophoblast and stem cell differentiation. We found DNA methylation was negatively associated with mRNA levels in both comparisons. Further De-methylation of Snail, Slug and MMP3 happened in IMR90 cells compared to iPS cells, whereas only Slug gene was de-methylated in the HTR8/SVneo cells compared with BeWo cells. This result suggested that the EMT of Stem cell mesenchymal differentiation was more complete than the EMT of trophoblast cells.

Conclusion: The present study is the first time to show Snail and Slug genes, master genes in EMT process, are mainly regulated by DNA methylation. Snail gene de-methylation and activation may play critical role in trophoblast EMT/invasion.

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The Antiphospholipid Antibodies (aPL)-Mediated Inhibition of Human Endometrial Endothelial Cells (HEEC) Angiogenesis. Is There a Role for Low Molecular Weight Heparins (LMWHs)? Silvia D'Ippolito,[1] Riccardo Marana,[1,2] Fiorella Di Nicuolo,[1] Roberta Castellani,[1] Manuela Veglia,[1] Chiara Tersigni,[1] John Stinson,[3] Giovanni Scambia,[1] Nicoletta Di Simone.[1] *[1]Department of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore; [2]ISI Istituto Scientifico Internazionale Paolo VI, Università Cattolica del Sacro Cuore; [3]LEO Pharma, Ballerup, Denmark.*

BACKGROUND: Antiphospholipid syndrome (APS) is characterized by vascular thrombosis and/or pregnancy morbidity and circulating aPL. A direct effect of aPL on trophoblast and HEEC has been proposed to explain the aPL-mediated pregnancy failure. Indeed, bound aPL induce trophoblast and HEEC dysfunction. APS patients can be treated with LMWH, which may act through mechanisms alternative to anticoagulation. We previously showed that LMWH reduces aPL binding to trophoblasts and restore cells functions. So far, however, no study has described its effects on endometrial angiogenesis. Aim of our research was to evaluate whether two LMWHs, tinzaparin and enoxaparin, have an effect on the aPL-inhibited endometrial angiogenesis. METHODS: We investigated: (i) *in vitro* HEEC angiogenesis through a Matrigel assay; (ii) VEGF secretion by ELISA; (iii)MMP-2 activity by gelatin zymography; (iv) Nuclear Factor-kB (NF-kB) DNA binding activity by colorimetric assay; (v) STAT-3 activation by a sandwich-ELISA; (vi) LMWHs effect in a murine model of *in vivo* angiogenesis. RESULTS: addition of LMWHs prevents aPL-inhibited HEEC *in vitro* and *in vivo* angiogenesis and is able to restore the aPL inhibited NF-kB and/or STAT-3 activity, the VEGF secretion and the MMP2 activity.

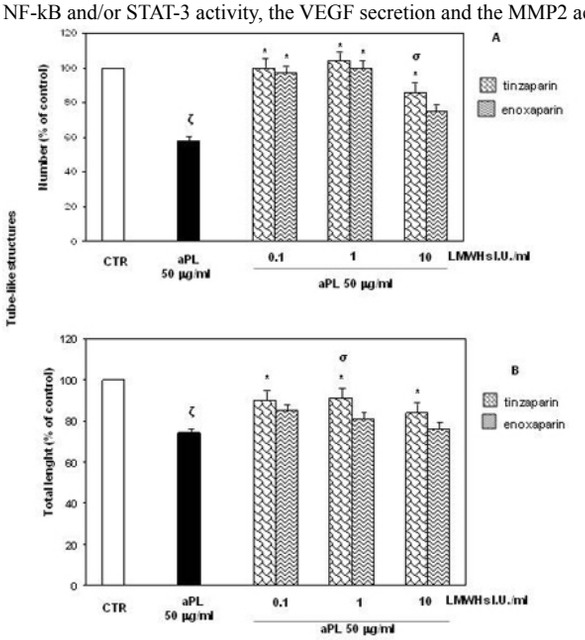


Figure 1. *In vitro* angiogenesis assay. Quantitative analysis of number (A) and total length (B) of tube-like structures after treatment with aPL (50 mg/ml) with or without tinzaparin or enoxaparin (0.1-10 IU/ml). Results are means ± SE of five experiments and expressed as % of control (CTR = 100). (CTR: untreated cells; ζ P<0.05 compared with CTR; *: P<0.05 compared with aPL; σ: P<0.05 enoxaparin vs tinzaparin).

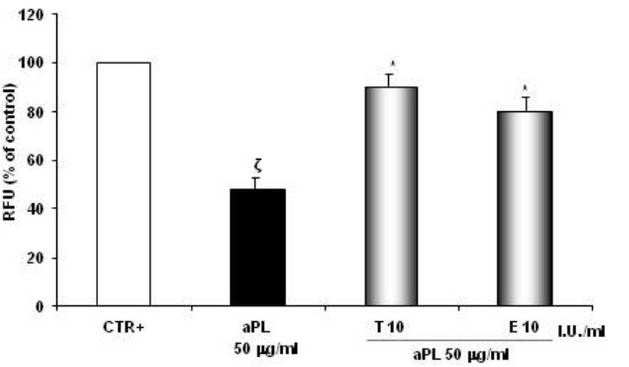


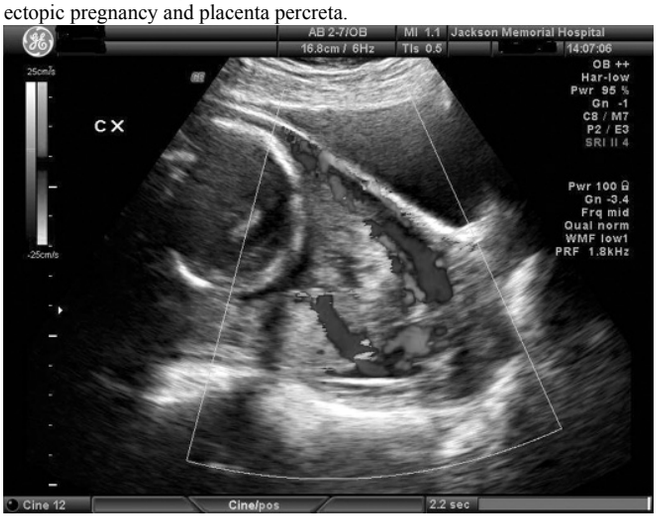
Figure 2. *In vivo* angiogenesis assay. Effects of aPL with or without LMWHs on angiogenesis process *in vivo*. Results are means ± SE (n = 5 mice per group) from three independent experiments and expressed as RFU (Relative Fluorescent Units), % of control. CTR+: positive control; T: tinzaparin; E: enoxaparin; I.U.: International Units; ζ P<0.05 compared with CTR; *: P<0.05 compared with aPL treatment.

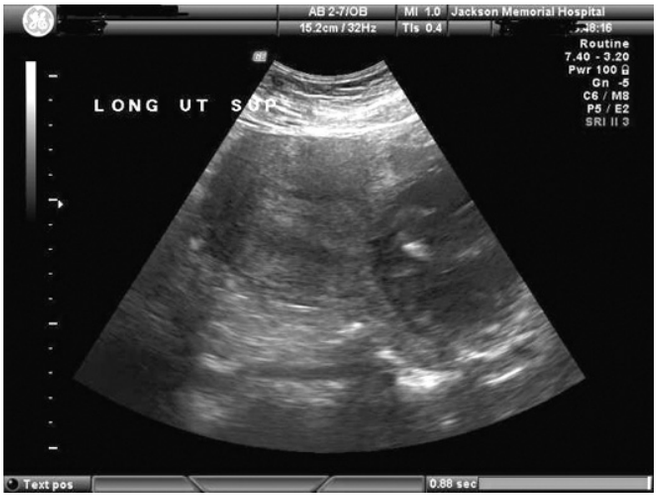
CONCLUSIONS: The demonstration of a beneficial role for LMWHs on the aPL-inhibited HEEC angiogenesis might provide additional mechanisms whereby this treatment protects early pregnancy in APS.

S-160

Conservative Multi-Modality Management of a Mid-Trimester Cervical Ectopic Pregnancy with Placenta Percreta Followed by a Successful Term Pregnancy. Diana P English, Salih Yasin, Fausto Andrade. *Obstetrics and Gynecology, University of Miami/Jackson Memorial Hospital, Miami, FL, USA.*

OBJECTIVE: To report the successful management of advanced cervical ectopic pregnancy with placenta percreta followed by a term pregnancy.
DESIGN: Case report. Setting: University tertiary care hospital.
MATERIALS AND METHODS: Single case report. The University did not require IRB approval for this case report according to the University of Miami Policies and Procedures on medical case reports.
PATIENT(S): A 31-year-old woman with a mid-second trimester cervical ectopic pregnancy and placenta percreta.





INTERVENTION(S): Ultrasound-guided injection of potassium chloride into fetal heart followed by systemic methotrexate of the order used to treat gestational trophoblastic disease, uterine artery embolism, removal of fetal bones, ligation of cervical branches of uterine arteries, foley catheter placement for tamponade and injection of prostaglandin PGF2α analogue into the cervix. The use of these modalities, ultimately achieved successful conservative management without significant hemorrhage.
MAIN OUTCOME MEASURE(S): Low maternal morbidity and successful conservative management with proven preservation of future fertility.
RESULT: The advanced cervical ectopic pregnancy was successfully treated conservatively with minimal patient morbidity and this patient went on to deliver a term fetus two years later
CONCLUSION: Conservative management of cervical pregnancy is now gaining acceptance and is the desired choice of therapy especially if the patient wishes to retain childbearing function. The relatively late gestational age in this case required the use of multiple therapeutic approaches for successful overall management with minimal morbidity.

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Gestational Protein Restriction Alters the Expressions of Genes Related to Trophoblast Cell Differentiation in Rat Placental Junctional Zone. Haijun Gao, Uma Yallampalli, Chandra Yallampalli. *Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*
Gestational protein restriction (PR) has deleterious effects on placental development, causing long-term metabolic and cardiovascular consequences in offspring. To date, the effects of nutritional manipulation on placental junctional zones have been largely ignored. The altered profiles of steroid hormones in response to PR support the hypothesis that PR impairs the trophoblast differentiation in both junctional (JZ) and labyrinth (LZ) zones. This study investigated expressions of marker genes for main types of trophoblasts in both JZ and LZ at mid and late pregnancy in rats fed with or without protein restriction. Pregnant Sprague Dawley rats were fed a normal (20% protein, control; n=10) or a low protein diet (6%, PR; n=10) from Day 1 of pregnancy until sacrificed at Days 14 and 18. LZ and JZ were dissected for genes expression analysis by Real-time PCR. The gender of placenta was determined by PCR on *sry* gene. The main findings include: 1) At Day 18 of pregnancy, the mRNA levels of *Esrrb* [marker for trophoblast stem cells (TS)] were increased by 2.9- and 2.0-fold ($P<0.01$) in PR female and male JZs, respectively. Similarly, elevated expressions of other TS markers *Id1* and *Id2* were seen in both PR female and male JZ ($P<0.01$); 2) At Day 18 of pregnancy, the mRNA levels of *Prl6a1* (marker for spongiotrophoblast in JZ) was decreased by 2.2- and 1.9-fold ($P<0.01$) in PR female and male JZs, respectively. Similarly decreased expression of *Ascl2* (marker for spongiotrophoblast in JZ) was shown in PR JZs at Day 18 of pregnancy; 3) At Day 14 of pregnancy, the mRNA levels of *Prl2c1* (marker for trophoblast giant cells in JZ) were decreased by 2.4-fold and 12.4-fold in PR female and male JZs, respectively ($P<0.001$). The decreased expression of *Prl2c1* also occurred in PR JZs at Day 18 of pregnancy; 4) Expressions of *Gjb3* (marker for glycogen trophoblast cells in JZ), *Gcm1* and *Gm52* (markers for syncytiotrophoblast cells in LZ) were not affected by PR at Days 14 and 18 of pregnancy; 5) At Day 18 of pregnancy, PR decreased the expression of *Ctsq* (marker for sinusoidal trophoblast giant cells in LZ) in both female and male LZ ($P<0.05$). These results indicate that PR may impair the differentiation of TS into spongiotrophoblast and trophoblast giant cells in JZ, and into sinusoidal trophoblast giant cells in LZ (Supported by National Institutes of Health grants R01HL102866 and R01HL58144).

S-162

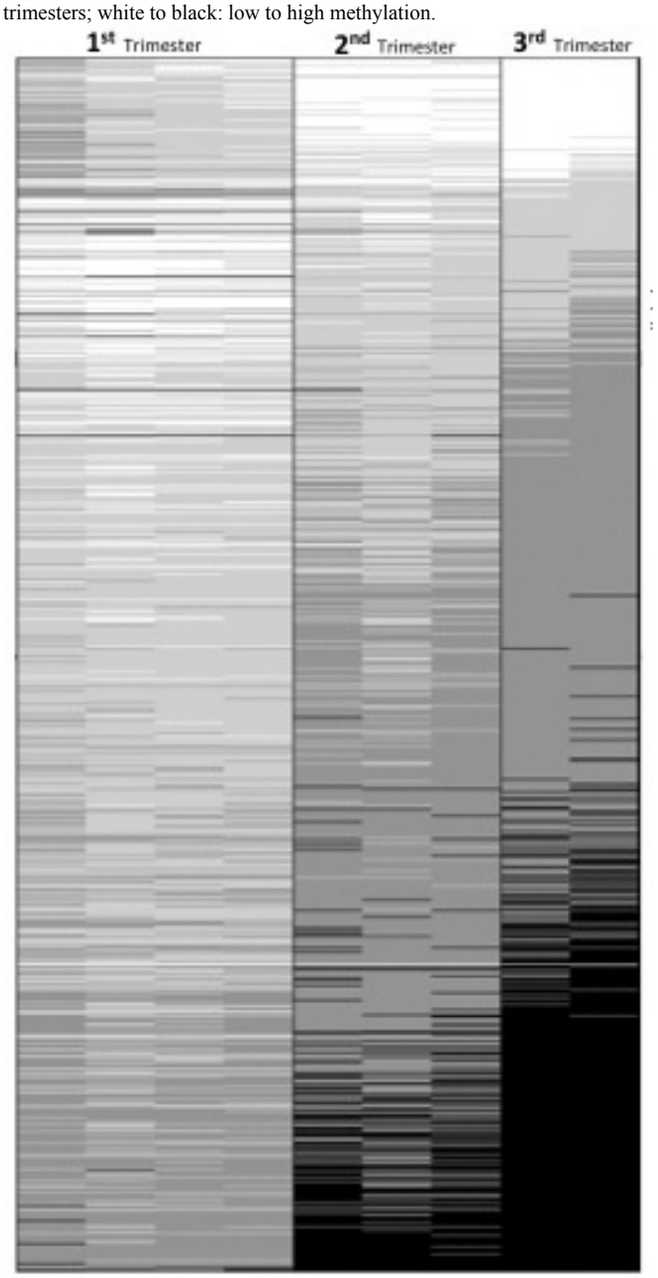
Characterization of Adhesion Molecule Expression of Trophoblast Cell Lines and Possible Implications for Trophoblast Homing to the Endothelium. Tanja Groten,[1] Ekkehard Schleupner,[1] Udo Markert,[1] Berthold Huppertz.[2] *[1]Obstetrics and Gynecology, University Hospital Jena, Jena, Germany; [2]Institute of Cell Biology, Histology & Embryology, Medical University of Graz, Graz, Austria.*
OBJECTIVES: Trophoblast homing to maternal spiral arteries is mandatory for successful placentation. Cell-cell adhesion molecules are regulating this process and adhesion molecule expression is altered in impaired placentation. We aimed to characterize cell-cell adhesion molecule expression on commonly used trophoblast cell lines in order to identify molecules involved in trophoblast-endothelial interaction.
METHODS: Expression of adhesion molecules like VCAM, PECAM, ICAM and selectin P and E and their corresponding ligands CD162 und CD147 were investigated in HUVEC and the trophoblast cell lines HTR-8, JEG-3, ACH3P and AC1M32.
RESULTS:
HUVEC HTR-8 JEG-3 ACH3P AC1M32
mRNA/Protein mRNA/Protein mRNA/Protein mRNA/Protein mRNA/Protein
ICAM-1 + / + +/ + +/ + +/ + +/ +
PECAM + / + -/ - -/ - -/- -/-
VCAM + / + +/ + -/ - -/- -/-
E-selectin + / + -/ - -/ - -/- -/-
CD147 + / + -/ - -/ - -/- -/-
P-selectin + / + -/ - -/ - -/- -/-
CD162 + / - +/ + -/ - -/- +/-
CONCLUSIONS: Cell-cell adhesion molecules mediating lymphocyte recruitment to the endothelium are possible candidates regulating trophoblast recruitment to maternal spiral arteries. HUVEC express ICAM and E-selectin, all trophoblast cell lines express the corresponding ligands ICAM and CD147. Furthermore, HUVEC are positive for VCAM and P-selectin mRNA. The corresponding ligands VCAM and CD162 are exclusively expressed in the extravillous cell line HTR-8. Thus, specific interaction between HUVEC and the extravillous trophoblast cells might be mediated by these adhesion molecules. This hypothesis is currently evaluated in co-culture experiments.

S-163

Human Placenta Undergoes Global DNA Methylation Modification during Normal Gestation. Sahar Houshdaran, Gabriel A Goldfien, Katherine Bianco. *Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA.*
Objective: Placenta is of utmost importance for intrauterine fetal development and growth. Epigenetic abnormalities may be at the root cause of dysregulation of placentation that can lead to adverse perinatal outcomes. However, global epigenetic modifications during normal gestation is not fully understood. Herein, we investigated global DNA methylation of normal placentation through the length of gestation to better understand the extent of epigenetic changes.
Methods: Basal plate biopsies (9 placentas) were collected from patients without any medical complications from first trimester to third trimester of the pregnancy. Specifically, from 5.1-11.3 weeks (n=4) for 1st trimester, 20.3-23.1weeks (n=3) for 2nd trimester, and from 36-39 weeks (n=2) for 3rd trimester. Bisulfite modified genomic DNA was hybridized to Illumina Infinium methylation that assays 27,578 CpG sites in the human genome. Statistical significance was set at P-value < 0.05 (Kruskal-Wallis Test) across trimesters and by comparing Median β-value difference >0.2 among trimesters.
Results: We observed distinctive methylation profiles between trimesters. We found 351 probes with less methylation in 1st compared to 2nd; 231 probes with less methylation in 2nd compared to 3rd; and 1213 probes in 1st compared to 3rd. Assessing gain of methylation showed a similar pattern: 171 probes more methylated in 1st vs. 2nd ; 65 probes in 2nd vs. 3rd; and 226 probes in 1st vs. 3rd. The greatest difference was between the 1st and the 3rd trimesters. While both gain and loss of methylation were observed from the transition of one trimester to the next, the majority of differentially methylated CpG sites gained methylation in the subsequent trimester suggestive of a gradual gain of methylation at specific loci during normal gestation.

Conclusion: Our data suggest that the placenta undergoes diverse DNA methylation changes during normal gestation and that specific epigenetic regions may regulate early development compared to late gestation.

Figure1. Probes with P <0.05 and median β-value difference >0.2 in all trimesters; white to black: low to high methylation.



S-164

The Golgi and Trans-Golgi Network Compartments a Dramaticaly Altered Following Cell Fusion. Gen Ishikawa,[1,2] Toshiyuki Takeshita,[2] William E Ackerman,[3] Dale Vandre,[1] John M Robinson.[1] *[1]Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA; [2]Obstetrics and Gynecology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan; [3]Obstetrics and Gynecology, The Ohio State University, Columbus, OH, USA.*

Objectives: Formation of syncytia from mononuclear cells leads to a number of changes in cellular physiology and function. We hypothesized that such changes will be reflected at the organelle level. Herein, we focus on alterations to the Golgi complex (GC) and trans-Golgi network (TGN) associated with BeWo cell fusion.

Methods: Cultured BeWo cells were used as surrogates for cytotrophoblasts and were induced to fuse by treatment with forskolin (FK). BeWo cells treated with FK for 24, 48, and 72 hours were compared to untreated control cells using immunofluorescence, and immunoblotting methods. Antibodies to the GC marker proteins GM130, giantin, and beta-COP were used along with TGN 46 and p230 for the TGN. In some experiments, JAR cells in the presence or absence of FK treatment were also used.

Results: In control cells (non-fused), the GC and TGN had a perinuclear distribution typical of cultured cells as determined by immunofluorescence microscopy. In contrast, fused cells had dramatically altered GC and TGN architecture, which were typically very enlarged and often formed giant GC and TGN structures. There was a strong association between the GC and TGN with nuclei in syncytial structures. The immunofluorescence staining patterns suggested that the GC and TGN had increased in amount when compared to control cells. This was further investigated using immunoblotting methods; a time-dependent increase in GC and TGN markers proteins was found in FK-treated fused cells.

Conclusions: We show that there is a dramatic alteration in the architecture of GC and TGN along with an increase in GC and TGN marker proteins in fused BeWo cells. We propose that this is due to increases in protein synthesis and subsequent post-translational processing occurring in syncytial structures. These results may have implications for increased protein trafficking and export necessary for syncytiotrophoblast function.

S-165

The Protein Deacetylase, Sirt1, Plays a Key Role in Trophoblast Differentiation and Placental Development. Pooja Iyer,[1] Matteo Moretto Zita,[1] Julia Peng,[1] Veronique Tache,[2] Michael McBurney,[3] Mana M Parast.[1] *[1]Pathology, UC San Diego; [2]Reproductive Medicine, UC Davis; [3]Medicine & Biochemistry, University of Ottawa.*

Aim: Appropriate differentiation of trophoblast is key for proper placental development and function, and in turn, for appropriate in utero fetal growth. Sirtuin-1 (Sirt1) is an NAD+-dependent protein deacetylase, shown to mediate nutrient deprivation-induced longevity in mammals through modification of both histones and other nuclear proteins, including the nuclear receptor PPARγ. Sirt1 is abundantly present in placentas of both mouse and human, including trophoblast, but its role in this organ is largely unknown. Sirt1-null mice are growth restricted and die in utero during mid-to-late gestation. The goal of this study was to investigate the role of Sirt1 in trophoblast differentiation, placental function, and fetal growth restriction.

Methods: Sirt1-heterozygous mice were bred, and pregnant dams sacrificed at various points during gestation (E13.5-E16.5). Embryos and placentas were genotyped and weighed; half of each placenta was processed for histology and half was snap-frozen for RNA isolation. Also, two wildtype (WT) and two Sirt1-null trophoblast stem (TS) cell lines were derived from E3.5 blastocysts, cultured, and differentiated per Tanaka et al. (Science, 1998). RNA was extracted from both placentas and TS cells using the mirVana kit (Ambion); qPCR was performed using trophoblast lineage-specific markers as previously described (Parast et al., PLoS One, 2009).

Results: Sirt1-null embryos were rare past E14.5; therefore, fetal and placental weights were compared at E13.5, prior to start of fetal resorption. Sirt1-null embryos and placentas were small compared to WT embryos (embryos: 70 + 22 mg vs. 114 + 33 mg, p=0.001; placentas: 51 + 12 mg vs. 61 + 12 mg, p=0.04). Morphologic assessment of Sirt1-null placentas showed a small, hypercellular labyrinth, compared to WT placentas. Sirt1-null TS cells showed significant reductions in both labyrinthine and trophoblast giant cell lineage-specific markers. Both Sirt1-null placentas and differentiated TS cells showed a significant decrease in PPARγ levels (placentas-3 fold; TS cells-9 fold).

Conclusion: Sirt1 is required for appropriate trophoblast differentiation and placental development. We hypothesize that the Sirt1-null fetal growth restriction phenotype is at least partially due to abnormal placental development and function.

S-166

Sphingosine-1-Phosphate (S1P) Inhibits Extravillous Trophoblast Migration Via S1P Receptor 2. Khiria Al-Saghir, Daman J Adlam, Melissa Westwood, Edward D Johnstone. *Maternal and Fetal Health Research Centre, University of Manchester.*

Introduction: Sphingosine-1-Phosphate (S1P) is a bioactive lipid that belongs to a signalling system recently identified through metabolomic analysis to be abnormal in pregnancy diseases that are associated with defective placental development. In other systems, S1P controls cell migration and can be stimulatory or inhibitory depending on which receptor is activated. Therefore in this study, we have characterised the S1P receptor repertoire and functional

response in extravillous trophoblast (EVT) to determine whether abnormal S1P signalling could contribute to abnormal placentation caused by deficient EVT migration.
Methods: The S1P receptor expression profile of primary first trimester trophoblast and Swan-71 cells, a well recognised model of EVT, was determined by RT-PCR and immunofluorescence. The effect of S1P (50nM-10μM) on Swan-71 migration was investigated using 8.0 μm cell culture inserts (BD Falcon) at 20% O_2 S1P receptor 2 (S1PR2) siRNA (200nM; Invitrogen) knockdown and the specific S1PR2 inhibitor JTE-013 (100nM) were used to demonstrate receptor activity.
Results: Swan-71 cells, like primary EVT, expressed S1P receptors 1-3 in similar abundance. S1P caused a marked reduction in Swan-71 migration at all concentrations, with maximal inhibition at 600nM (65±11%, n=6; $p<0.05$, Kruskal-Wallis). siRNA-mediated knockdown of S1PR2 expression (70% reduction in mRNA) prevented the inhibitory effect of S1P. Incubation of cells with JTE-013 had a similar effect, confirming the importance of this receptor in the negative regulation of trophoblast migration.
Conclusion: This study demonstrates that although EVT express three S1P receptor isoforms, signalling is predominantly through S1PR2 and thus S1P is a potent inhibitor of EVT migration. Further investigation of the relationship between S1P signalling and EVT migration in normal placental development and pregnancy disease is required however, these data suggest that shifting the balance of S1P receptor activation might provide a mechanism for improving impaired trophoblast migration.

S-167

Early Placental Gene Expression Using RNA-seq. Jennifer R King,[1] Sue A Ingles,[2] Ian B Tilley,[1] Vasu Punj,[2] Melissa L Wilson.[3] *[1]Department of Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA; [2]Department of Preventive Medicine and OB/GYN, University of Southern California, Los Angeles, CA, USA; [3]Department of OB/GYN and Preventive Medicine, University of Southern California, Los Angeles, CA, USA.*
Background: The placenta is the principal metabolic, respiratory, excretory and endocrine organ for fetal life. Gene expression in chorionic villi during early gestation regulates placentation, and several common obstetric diseases are associated with abnormal or impaired placental invasion. However, molecular mechanisms for normal and abnormal placentation have not been fully delineated.
Methods: In the present investigation, we sequenced complementary cDNA from placenta villi obtained at 7, 8, 9, and 15 weeks gestation, using massively parallel next-generation sequencing (RNA-seq). RNA-seq allows examination of global gene expression, with quantitative measurement of expression levels of genes and their transcripts. We tested for differential expression across gestational age.
Results: We obtained more than 40 million 100 bp paired-end reads from each sample and identified more than 36,300 previously annotated transcripts from more than 15,000 genes. Compared to baseline (7 weeks), approximately 29% of transcripts at each time point were more than two-fold up-regulated. The number of transcripts down-regulated by more than two-fold increased from 8% at 8 weeks to 14% at 9 weeks and 20% at 15 weeks gestation.
Conclusions: Early placentation involves a complex mechanism of altered gene expression. Further identification of these regulated gene transcripts will serve as a foundation for understanding normal placental function and allow for future comparison with pregnancies complicated by obstetric disease.

S-168

The Role of ΔNp63α in Human Cytotrophoblast Stem Cell Proliferation and Lineage Specification. Yingchun Li, Matteo Moretto Zita, Anna K Wakeland, Mana M Parast. *Pathology, School of Medicine, University of California, San Diego, CA, USA.*
The placenta is a transient organ, necessary for proper fetal development. Its main functional component is the trophoblast, which is derived from extraembryonic ectoderm. Little is known about the trophectoderm in the human embryo, as human trophoblast stem cells have yet to be isolated and characterized. p63, a member of the p53 family of nuclear proteins, has been shown to be involved in stem cell maintenance in skin and other stratified epithelia. We have previously identified p63 as a marker of proliferative trophoblast in human placental tissues. Specifically, based on immunohistochemical staining, p63 is expressed in all Ki67-positive trophoblast, including cytotrophoblast in the floating chorionic villi and cytotrophoblast cell columns in anchoring villi; however, it is absent in differentiated trophoblast, both syncytiotrophoblast of the chorionic villi and the invasive extravillous trophoblast in the placental bed. Here, we have further studied its role in trophoblast proliferation, using the human trophoblast cell lines, BeWo and JEG3, and primary cytotrophoblast (CTB) isolated from first trimester or term placental tissue. BeWo cells can be induced to differentiate into syncytiotrophoblast by treatment with forskolin, while primary CTB differentiate spontaneously to syncytiotrophoblast over a 7-day period in culture. We showed that p63 expression in all these cells is limited to the ΔNp63α isoform (from here on referred to as p63) and is lost as the cells (both BeWo and primary CTB) differentiate into syncytiotrophoblast. We generated lentivirus, both for overexpression and downregulation of p63. Overexpression increased, while downregulation decreased, trophoblast proliferation, as measured by BrdU uptake assay. In addition, when cells were cultured under low oxygen tension, conditions which promote CTB proliferation and inhibit syncytiotrophoblast differentiation, p63 expression is enhanced. Finally, p63 expression was induced in human embryonic stem cells (hESC, WA09/H9 line), following differentiation into trophoblast lineage by short-term treatment with BMP4, and was further enhanced when BMP4 treatment was performed under hypoxia. Downregulation of p63, using shRNA lentivirus, inhibited BMP4-induced trophoblast differentiation of hESCs. Taken together, our results indicate that p63 plays an important role in both trophoblast proliferation and lineage specification.

S-169

The Role of Toll-Like Receptors in the First Trimester of Pregnancy: Comparison of Their Expression with the Immunohistochemistry Method between Normal and Pathological Implantation. Mona Mansour, Gaetano Bulfamante, Laura Avagliano, Maria Chiara Autuori, Anna Maria Marconi. *Obstetrics and Gynaecology, San Paolo H. University of Milan, Milan, Italy.*
Introduction : The attention of researchers in the recent years has focused on the critical role of the innate immunity in fetal implantation and the involvement of Toll Like Receptors (TLRs) in the pathogenesis of several disorders of pregnancy.
Objective: To verify with the immunohistochemistry method the role of the 10 "TLRs" at the level of the trophoblast (T) and implantation vessels (IVs), in both physiological and pathological implantations.
Materials and methods: 400 women were enrolled during their first trimester of pregnancy between years 2008 and 2009.They have been subjected to Dilatation and Curettage.360 had a diagnosis of spontaneous abortion(SP) and 40 have been represented by women who had voluntary interruption of pregnancy (VIP),who have been enrolled as controls.The SP group was then divided into 2 other groups: the first represented by pathological implantation cases and the second with a normal one.Finally 20 cases in Group I (SP) and 10 in group II (VIP) were finally eligible for the study. Exclusion criteria were abnormal karyotype and cases where at the site of implantation all components of the T cells and/or IVs were not available.
Results: Among the different cell populations of the T,TLRs were expressed only in the villous cyto-T and the extravillous-T. In contrast, the syncytio-T did not express these receptors: this suggest that the placenta functions as a highly specialized barrier protecting the developing embryo against pathogens.TLRs 1,2,4,5 were particularly expressed in the endovascular T. At the level of utero-placental vessels it was observed that TLR-5 and TLR-10 were well expressed in normal IVs and non implantations ones, while absent in non modified IVs.
Conclusions: The expression of TLRs in the various types of the T is quite different. In consideration of the specificity of each receptor-binding ligand, it is likely that these differences express different functions of each receptor. To better understand the role of TLRs in the implantation, other researchs are essential for evaluating their expression in habitual abortions sine causa and their role in the process of apoptosis and hereby possible involvement in the IUGR.One potential future study could be the use of TLRs agonists (or antagonists) as a treatment of certain disorders of pregnancy.

S-170

The 2-Arachidonylglycerol-Metabolising Enzyme (MAGL) and Receptor Targets (CB1, CB2) Are Up-Regulated in Miscarried Trophoblast Tissues. Timothy H Marczylo, Amy Gorman, Katerina Bambang, Tulay Karasu, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences Section, CSMM, University of Leicester, United Kingdom.*
Introduction: Anandamide (AEA) is significantly up-regulated in plasma and the enzyme responsible for degradation of AEA, fatty acyl amide hydrolase (FAAH) is downregulated in white blood cells of pregnant women that go on to miscarry. The structurally related 2-arachidonylglycerol (2AG) is a more potent ligand for cannabinoid receptors (CB1, CB2) and is synthesized by different enzymes to AEA. 2AG is synthesized by diacylglycerol lipase (DAGL; two

isoforms α and β) and degraded by monoacylglycerol lipase (MAGL). We investigated transcript levels of these receptors and enzymes in trophoblast tissue following miscarriage and surgical termination of pregnancy (STOP).
Methods: Transcript levels were investigated by quantitative real-time PCR (qRT-PCR) in 12 trophoblasts samples (5 spontaneous miscarriages, 7 STOP). Suitable housekeeping genes (18S, ACTB, ATP5B, B2M, CYC1, EIF4A2, GAPDH, SDHA, RPL13A, TOP1, UBC and YWHA2) were investigated and the optimal 2 genes selected by geNorm and Normfinder analyses.
Results: Optimal housekeeping genes were ATP5B and UBC. Transcripts for CB1, CB2, MAGL and both α and β DAGL were present in all trophoblast samples and were higher in miscarriage samples compared with STOP but only CB1 reached statistical significance (P<0.001).
Conclusions: Endocannabinoids play an important role in pregnancy maintenance. Over-expression of CB1 may lead to pregnancy termination.

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The Role of Autophagy for EVT Invasion and Vascular Remodeling in Preeclampsia. Akitoshi Nakashima, Tomoko Shima, Kumiko Inada, Shigeru Saito. *Obstetrics & Gynecology, University of Toyama, Toyama, Japan.*
Autophagy is an intracellular bulk degradation system through which cytoplasmic components are degraded in lysosomes. The primary roles of autophagy are to maintain intracellular homeostasis under starvation. Extravillous trophoblast (EVT) cells characterize the invasion of the maternal decidua under low oxygen at the feto-maternal interface for establishing successful pregnancy. Shallow EVT invasion is one of the critical causes of preeclampsia. So far, it is unclear why EVT deeply invade to the maternal decidua under hypoxia. Here, we examined whether autophagy play a role for EVT invasion or vascular remodeling. Two autophagy-defect EVT cell lines, HTR8- and HchEpC1b (Hch)-Atg4BCA, were established by Atg4B-mutant gene transfer. By using the invasion chamber assay, the number of invaded EVT which lack autophagy were significantly lower than that of control cells under hypoxia (p=0.0001). We further analyzed the vascular remodeling of EVT cell line by the assay co-cultured with EVT cell line and HUVEC on matrigel. The average area of tube formation by the Hch-wild type were significantly higher than those by the Hch-Atg4BCA (p=0.005). These data showed that autophagy played an important role in EVT functions. We further examined whether preeclampsia related factors (TNF-a, TGF-b, soluble endoglin: sEng and soluble Flt-1) affect autophagy in EVT. The incidence rate of autophagy in EVT under hypoxia was significantly decreased to 50% by sEng treatment (100ng/ml, p<0.01), but not by other factors. Additionally, both the invaded EVT cell number and the vascular formation were significantly suppressed by sEng treatment in Hch-wild type cells under hypoxia (p<0.01), but not in Hch-Atg4BCA cells. Soluble endoglin might contribute to the inhibition of hypoxia-induced autophagy, resulting poor placentation in preeclampsia. We next examined the expression of p62, the selective degraded substance by autophagy, as an autophagy inhibition marker. In vitro assay showed that this molecule was degraded by autophagy in Hch-wild type cells under hypoxia, but not in autophagy-defect Hch cells under hypoxia. In placental bed biopsy samples obtained from patients, the number of p62-positive EVT cells in preeclampsia was significantly higher than that in normal pregnancy (p<0.01), suggesting the autophagy was suppressed in EVT cells in preeclampsia. This is the first report that autophagy in EVT contributes to the pathophysiology of preeclampsia by sEng.

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Do Trophoblast Progenitor Cells Facilitate the Placental Response to Stress during Pregnancy in the Mouse? Christina Schweitzer, Bryony V Natale, David RC Natale. *Comparative Biology & Experimental Medicine, University of Calgary, Calgary, AB, Canada.*
OBJECTIVE: To investigate the contribution of trophoblast progenitor cells to adaptation of the mouse placenta in response to stress during pregnancy.
METHODS: Using a previously described model of reduced uteroplacental perfusion pressure (RUPP) in pregnant mice, uterine arteries were ligated at embryonic days (E) 14.5 and 16.5 to reduce blood flow to the placenta and developing fetus. Feto-placental units were dissected from RUPP or sham-operated controls two days following ligation. Fetuses and placentas were weighed, fixed, and placentas sectioned for histological staining, immunohistochemistry and in situ hybridization.
RESULTS: Uterine artery ligation at E14.5 and E16.5 resulted in different effects on fetal and placenta weights. Uterine artery ligation at E14.5 resulted in no change in average embryo weight but a 10% increase in average weight of placentas by E16.5 (n=5 litters, p < 0.01). Uterine artery ligation at E16.5 resulted in a 13% reduction (n=5 litters, p < 0.01) in average embryo weights but no change in placental weights by E18.5. Histological and molecular analyses were conducted on tissue sections from these placentas to assess changes in proliferation and the expression of markers of trophoblast sub-types. Importantly, we noted a significant increase in proliferating trophoblast cells at a time in gestation when there are normally very few. Phospho-histone H3 and cytokeratin double fluorescent immunostaining localized these cells only within the labyrinth layer of placentas subjected to uterine artery ligation at E14.5. When examined for the expression of molecular markers of different trophoblast sub-types by in situ hybridization, an increased expression of ascl2 messenger RNA was observed, also a marker of undifferentiated trophoblast progenitor cells. In addition, the architecture of the placentas in this group was different, displaying an increased cell density as well as increased numbers of spongiotrophoblast and glycogen trophoblast cells within the labyrinth, which were localized close to the chorionic plate.
CONCLUSIONS: Our results suggest that the ability of and mechanisms by which the mouse placenta responds to stress during pregnancy is dependent upon the timing of the stress. Furthermore, it appears to involve the recruitment of trophoblast cells to proliferate and differentiate in order to facilitate the remodeling of the labyrinth layer.

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Decidualization Conditions Promote Attachment and Invasion of Trophoblast-Like Jar Spheroids to a Novel Bioengineered Three-Dimensional (3D) Human Endometrial Culture System. Hai Wang,[1] Silvina Bocca,[1] Sandra Anderson,[1] Jose Horcajadas,[2] Sergio Oehninger.[1] *[1]Dept of OB/GYN, The Jones Institute for Reprod Med; [2]Araid at I+CS, Zaragosa, Spain.*
Objective: Endometrial decidualization is a *conditio sine qua non* for normal embryonic implantation. This process can be recapitulated in cell cultures of enriched endometrial stromal cells, is activated by cAMP, and is augmented by progesterone. Here, we aimed to (i) develop a human implantation model including an endometrium-like 3D culture system and trophoblast-like Jar spheroids, and (ii) investigate the effect of decidualization conditions on the attachment and invasion of Jar spheroids into the 3D culture system.
Methods: The endometrium-like 3D culture system was constructed with a fibrin-agarose gel matrix, with epithelial cells (Ishikawa) seeded on top, and stromal cells (HESC) residing within the 3D matrix (Wang et al, SGI 2011). Trophoblast-like spheroids (100-150 µm, demonstrated to secrete hCG) were constructed using Jar cells. After 3 days of culture, the 3D constructs were (i) treated with 17β estradiol (E_2, 10^{-8} M) and medroxyprogesterone acetate (MPA, 10^{-6} M) for 2 days, and E_2 (10^{-8} M) and MPA (10^{-6} M) combined with cAMP (0.5mM) for 1 additional day, after which Jar spheroids were transferred onto its top and co-cultured for 25 min for attachment studies; or (ii) co-cultured with Jar spheroids in the presence of E_2 (10^{-8} M) + MPA (10^{-6} M) for 2 days, and then E_2 (10^{-8} M) + MPA(10^{-6} M) + cAMP (0.5mM) for 1 additional day, for Jar spheroid invasion studies. The attachment rate was quantified by a centrifugal force-based adhesion assay. The invasion depth of Jar spheroids (loaded with CMFDA) was analyzed with Z-images captured by confocal microscopy.
Results: The attachment rate of Jar spheroids to the 3D culture system in the treatment group was significantly higher than that in control group (65.7 ± 4.0% vs. 50.0 ±3.9%, P<0.05), but only slightly higher than that of Jar spheroids to an epithelial cell monolayer under E_2+MPA+cAMP treatment (65.7 ± 4.0% vs. 59.2 ±3.1%, P>0.05). The invasion depth of Jar spheroids was significantly higher under E_2+MPA+cAMP treatment than in controls (137.5±6.1µm vs. 102.7±4.9µm, P<0.05).
Conclusions: The newly developed human 3D endometrial culture/trophoblast-like Jar spheroid system responds to decidualization signals, favoring embryonic attachment and invasion, and constitutes a valid model to examine certain aspects of human implantation *in vitro*.

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hCG Activates Endothelial Cell Proliferation Via Signaling Loop Involving FGF and p44 MAPK. Anna Polec,[1] Tom Tanbo,[2] Anne Eskild,[1] Peter Fedorcsak.[2] *[1]Department of Obstetrics and Gynecology, Akershus University Hospital, Lørenskog, Norway; [2]Women and Children's Division, Oslo University Hospital, Oslo, Norway.*
INTRODUCTION
Placental angiogenesis is essential for fetal growth and development. In addition to the ubiquitous angiogenic factors like basic fibroblast growth factor (bFGF), pregnancy-specific factors like human chorionic gonadotropin (hCG) are suggested to play a role in this process. In this study we studied effect of hCG and bFGF on endothelial cell proliferation in vitro.

METHODS
Human umbilical vein endothelial cells (HUVEC) were seeded on gelatin-coated flasks and incubated in HUVEC complete medium. Cells were grown to confluence and sub-cultured for proliferation and cell signaling assays. The cells were incubated for 2 days with dilution series of hCG and/or epidermal growth factor (EGF), bFGF, and incorporation of radiolabelled thymidine in proliferating HUVEC cells' DNA was assessed. Cells at confluent stage were serum-starved and exposed to hCG and EGF, bFGF or both for various incubation times (15', 30', 60' and 120'). After exposure cells were fixed, permeabilized, and labeled for activated p44/42 mitogen-activated kinase (MAPK). Samples were examined by flow cytometry.
RESULTS
hCG alone had no effect on endothelial cell proliferation compared to control. FGF increased cell proliferation dose-dependently. Simultaneous addition of FGF and hCG exerted additive effects on cell proliferation. Cells exposed to both hCG and bFGF displayed increased activation of p44/42 MAPK compared to the effects of hCG or bFGF alone.
CONCLUSIONS
Activation of p44/42 mitogen-activated kinase (MAPK) pathway by growth factor receptors in endothelial cells is related to cell proliferation. We found that hCG and bFGF additively activate p44 MAPK suggesting that these two factors can operate through converging signaling pathways. There could exist potential signaling cross-talk between the hCG and FGF in MAPK pathways, explaining a synergistic effect of hCG and FGF on the proliferative response of cells.

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Relaxin Stimulates Trophoblast Invasion In Vitro. Emiel D Post Uiterweer,[1,2] Diana Herrera,[2] Jonathan T McGuane,[2] Laura J Parry,[3] Kirk P Conrad.[2] *[1]Department of Obstetrics, University Medical Centre Utrecht, Netherlands; [2]Departments of Physiology and Functional Genomics, and of Obstetrics and Gynecology, University of Florida, USA; [3]Department of Zoology, University of Melbourne, Parkville, Australia.*
Introduction: Trophoblasts are placental cells that invade the uterus and spiral arteries in normal pregnancy. This results in transformation of the spiral arteries into low-resistance, high-capacitance vessels, ensuring unimpeded blood flow to the villous placenta. Aberrations in this process are associated with hypertensive disease later in pregnancy. Endothelin-1 and the endothelin B (ET_B) receptor are known to promote migration of both trophoblasts and endothelial cells via nitric oxide (NO), which is a vasodilator. Our previous research shows that the pregnancy hormone relaxin activates this pathway in endothelial cells via the RXFP-1 receptor, with vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and matrix metalloproteinases (MMP-2 and MMP-9) as upstream intermediaries.
Objectives: To establish if (1) the molecular constituents of the 'relaxin vasodilatory pathway' are expressed in extravillous trophoblast cells, and (2) to test the hypothesis that recombinant human (H2) relaxin (rhRLX) stimulates invasion of these cells.
Methods: The HTR-8/SVneo first trimester extravillous trophoblast cell line was used. Gene and protein expression were determined by RT-PCR and immunofluorescence confocal microscopy, respectively. The effect of rhRLX (3, 30 and 300 ng/ml) or vehicle on trophoblast invasion was examined using a FluoroBlok Matrigel invasion assay. The MMP inhibitor GM6001 (25 μM) was used to block rhRLX-stimulated invasion. Analysis was performed by SYTOX Green staining of invaded cells, and automatic counting of nuclei with ImageJ Software.
Results: HTR-8/SVneo cells express the molecular components of the 'relaxin vasodilatory pathway' including RXFP-1, which was localized to the trophoblast plasma membrane. In addition, the cells expressed genes of extravillous trophoblast origin – aromatase, placental alkaline phosphatase, and HLA-G – thus confirming their identity. rhRLX (3, 30 and 300 ng/ml) significantly stimulated invasion in a dose-dependent fashion (1-way ANOVA $p<0.001$). GM6001 inhibited rhRLX-stimulated, but not baseline (vehicle) invasion.
Conclusion: Relaxin stimulates trophoblast invasion, possibly through the same molecular pathway by which relaxin induces NO production in endothelial cells.

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Microarray Analysis of Fetal Cells in the Pregnant Mouse Lung Reveals Expression of Trophoblast-Specific Genes. Stephanie Pritchard,[1] Heather C Wick,[2] Kirby L Johnson,[1] Diana W Bianchi.[1] *[1]Mother Infant Research Institute, Floating Hospital for Children at Tufts Medical Center, Boston, MA, USA; [2]Department of Computer Science, Tufts University, Medford, MA, USA.*
Over 90% of women experience at least one pregnancy during their lifetime. Cells from each fetus are retained in maternal organs for decades postpartum. We sought to identify the type(s) of cells in this microchimeric population, as their phenotype could determine their effect on maternal health. We hypothesized that gene expression data could be used to elucidate fetal cell type in the maternal lung.
C57BL/6J females were mated to males homozygous for the green fluorescent protein (*gfp*) transgene. Pregnant mice (n=7) were sacrificed on day e18. GFP+ fetal cells were flow-sorted from single cell suspensions of maternal lung. cDNA was amplified using the WT-Ovation One Direct Amplification kit (NuGEN). cDNA was hybridized to Affymetrix Mouse 430 2.0 arrays. Three types of analyses were performed: 1) genes statistically significantly differentially up-regulated in fetal cells compared to maternal lung cells ($p<0.05$); 2) genes in the top 10% of those expressed; and 3) genes with a present call on all chips. Genes common to least two of the lists were selected for further investigation. We used this combined analysis to reduce bias and diminish the incidence of false positives. The BioGPS Gene Atlas was used to identify expression patterns. Genes were considered tissue-specific if they were expressed in only one tissue above 30 multiples of the median or if the expression in a tissue was at least 50-fold greater than any other tissue.
Gene expression information was successfully obtained from as few as 20 cells. 385 genes met the criteria above; 33 were tissue-specific. Fetal cells in the maternal lung were found to express several trophoblast-specific genes such as *psg18, cathepsin J, cathepsin Q, prl2b1* and *prl3b*. Other genes important for placental development and invasion were also expressed, including *peg10, ahnak, dusp1, runx1, uteroglobin, notch2* and *jagged1*.
These unexpected results suggest that some of the fetal cells present in the murine maternal lung are trophoblasts. Although trophoblasts are found in human maternal lung during pregnancy, their presence in the lungs of other species has not been previously demonstrated. The presence of trophoblasts in the lungs of mouse models may facilitate further study of their potential role in establishing tolerance to the fetus.

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Focal Adhesion Kinase Regulates AP-1 Factor Expression and Invasion in Trophoblast Cells. Stephen J Renaud, MA Karim Rumi, Michael J Soares. *Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS, USA.*
Trophoblast invasion and subsequent spiral arteriole remodeling ensures that a sufficient supply of maternal blood is available for the conceptus, and is an essential component of placental development. Aberrant trophoblast invasion has been linked with various pregnancy complications including pre-eclampsia, intra-uterine growth restriction, and the placenta cretas. Here, we present data obtained from human trophoblast cell lines indicating that focal adhesion kinase (FAK), a non-receptor tyrosine kinase predominantly localized to focal adhesions, regulates AP-1 factor expression and motility of trophoblast cells. FAK and phosphorylated FAK were detected at high levels in the immortalized human first trimester extravillous trophoblast cell lines HTR-8/SVneo and Swan-71, and at lower levels in a choriocarcinoma cell line (Jeg-3). Adhesion was a requirement for FAK activation. Suspended cells did not express activated FAK, reinforcing the importance of integrin signaling for FAK activity. To determine the role of FAK in trophoblast cell invasion, cells were treated with the FAK-specific inhibitor PF573228 or transduced with lentivirus containing PLKO vectors encoding short hairpin (sh)RNA for FAK. Treatment of cells with vehicle (DMSO) or PLKO vectors encoding shRNA for non-mammalian targets were used as controls. FAK activation was substantially reduced in cells after PF573228 treatment and after infection with lentivirus containing FAK-specific shRNA. Decreased FAK phosphorylation was associated with reduced invasion of all trophoblast cell-types as assessed by Matrigel invasion assays. Moreover, FAK-knockdown in HTR-8/SVneo and Swan-71 trophoblast cells caused decreased expression of the AP-1 transcription factor JUN, and increased expression of FOSB and FOS. Since Jeg-3 trophoblast cells expressed the lowest levels of active FAK, these cells were selected for transfection of a vector encoding a constitutively active FAK. Jeg-3 trophoblast cells ectopically expressing FAK exhibited substantially elevated levels of JUN and reduced levels of FOSB and FOS, concomitant with increased invasion through Matrigel. In conclusion, FAK is a key determinant of trophoblast cell

invasion, and may regulate invasion-promoting genes at least partly through modifying the expression of AP-1 factors. (Supported by the Canadian Institutes of Health Research, Lalor Foundation, and NIH HD20676.)

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Phosphatidylserine Externalization Does Not Occur during Primary Trophoblast Fusion. Meghan R Riddell,[1] Bonnie Winkler-Lowen,[2] Sandra T Davidge,[1,3] Larry J Guilbert.[2] *[1]Physiology, University of Alberta, Edmonton, AB, Canada; [2]Medical Microbiology and Immunology, University of Alberta; [3]Obstetrics & Gynecology, University of Alberta.*

Introduction: The villous placenta contains the non-proliferative, multinucleate syncytiotrophoblast (ST), and the proliferative, mononucleate cytotrophoblast (CT). The ST is maintained exclusively through fusion and differentiation of the underlying CT. The molecular signals and mechanisms controlling trophoblast fusion remain to be fully elucidated, but it has previously been shown using trophoblastic cell lines that widespread externalization of the phospholipid phosphatidylserine (PS) occurs during fusion and is maintained in the multinucleated state. PS is normally localized to the inner leaflet of the plasma membrane, and this conformation is actively maintained by the cell. PS externalization is a hallmark of early apoptosis, but has also been shown to occur during cell-cell fusion in both myoblast formation and macrophage fusion. The widespread and extended nature of PS previously observed in trophoblastic cell lines is not seen in other fusing cells, where PS externalization is limited to the sites of membrane fusion and membrane asymmetry is re-established after fusion has completed. PS externalization has not previously been examined in primary trophoblasts. In this study we examined PS externalization using isolated primary CT to examine the pattern of PS externalization observed during primary cell fusion.

Methods: Primary CT were isolated from 39 week normal term placentas delivered by caesarean section. CT were then plated with or without the cell permeant cAMP analog Br-cAMP for 24-72hrs. PS externalization was examined using Annexin V-FITC binding by immunofluorescent microscopy.

Results: There were no differences in the percentage of Annexin V positive cells between Br-cAMP stimulated cells and medium controls despite a nearly 50% increase over medium alone in the number of multinucleated cells by 72 hrs of Br-cAMP treatment. The percentage of cells with externalized PS peaked at ~10% in Br-cAMP stimulated cells after 24 hours in culture, and decreased to ~5% by 72 hours.

Significance: Phosphatidylserine externalization does not occur in the same volume or temporal pattern in primary trophoblasts compared to trophoblastic cell lines. Thus caution must be used when interpreting data that utilizes trophoblastic cell lines to examine the role of PS in trophoblast fusion.

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LIN28A Regulates Syncytialization and hCG Production in Human Trophoblast Cells. Jill L Seabrook, Jeremy D Cantlon, Erin E Soisson, Colin M Clay, Russell V Anthony, Quinton A Winger. *Department of Biomedical Sciences, Colorado State University.*

Preeclampsia (PE) and intrauterine growth restriction (IUGR) are associated with abnormal placenta development and function. A vital component of placenta development is the formation of the syncytiotrophoblast (ST) layer, which functions as the feto-maternal interface. The ST is maintained throughout pregnancy via continual cytotrophoblast (CT) differentiation and fusion. In addition to modulating metabolic exchange, the ST layer produces human chorionic gonadotropin (hCG), which is required for pregnancy recognition, trophoblast invasion and vascular remodeling. Placental explants cultured from PE and IUGR pregnancies have lower CT fusion indices, fewer nuclei per ST, and lower hCG secretion. Therefore, understanding the mechanisms controlling CT to ST differentiation is an important approach for developing early diagnostics for placental disease. LIN28A, an RNA binding protein, is detected in pluripotent cells and decreases with differentiation. In addition to inhibiting *pre-let-7* miRNA maturation, LIN28A selectively enhances translation in embryonic stem cells. We previously reported that *LIN28A* mRNA decreased with forskolin treatment, suggesting that fusion results in a higher degree of differentiation. In the current study we investigated how LIN28A regulates CT to ST differentiation. LIN28A expression was confirmed in ST and CT cells from human first trimester placental villi, and is highly expressed in ACH-3P cells, a first trimester trophoblast cell line. We constructed LIN28A-knockdown (KD) ACH-3P cells by infecting them with lentivirus expressing shRNA designed to target *LIN28A* mRNA. Knockdown resulted in a 56% reduction of LIN28A protein and mRNA. LIN28A-KD cells were treated with forskolin, and the syncytialization response was assessed. Basal levels of β-hCG (CGB) secretion were 6-fold higher in LIN28A-KD cells compared to controls ($P < 0.01$). Forskolin treatment resulted in 4-fold higher levels of CGB secretion ($P < 0.01$), and a 2-fold increase in α-subunit transcription compared to control cells ($P < 0.01$). LIN28A-KD cells had 3-fold higher levels of *syncytin-1* mRNA ($P < 0.01$) and a higher spontaneous fusion index compared to control cells. These data suggest that LIN28A has an inhibitory effect on key mechanisms regulating ST differentiation and fusion as well as hCG secretion, and that deregulation of LIN28A may contribute to the development of placental pathology.

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First Trimester Placental Explants Cultured under Hyperoxic Conditions Show Elevated PlGF Release but Are Otherwise Resistant to Changes in Oxygen Levels. Richard T Blankley, Elizabeth Cowley, Sylvia Liu, John D Aplin, Colin P Sibley, Jenny E Myers. *Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Background

We hypothesised that poor trophoblast invasion and hence ineffective plugging of maternal spiral arteries may lead to the premature flow of oxygenated blood into the intervillous space, which in turn may be a key pathological event in the later development of pre-eclampsia and/or IUGR. A number of proteins are altered in first trimester serum from women who go on to develop these pathologies. Our aim was to test if placental release of such proteins is affected by oxygen levels.

Methods

Placental villous explants were established from tissue obtained from termination of pregnancy procedures (n=6, all 7-9weeks gestation). Explants were pre-conditioned at 1% oxygen before being cultured at 1%, 6% or 20% oxygen in serum-free medium for 48h with a replacement of medium at 24h. PlGF, hCG, LDH, Activin A, hPL and Ang2 were measured in the conditioned media using commercial ELISAs.

Results

At the 24h time point there were no significant differences in the concentration of any measured proteins between the different oxygen tensions; PlGF (mean, SEM in pg/ml): 1% (51.8, 8.0), 6% (49.8, 2.9) and 20% (66.8, 6.3). At 48h the conditioned medium concentration of PlGF was significantly (Kruskal-Wallis one-way ANOVA) elevated at higher oxygen tensions (mean, SEM in pg/ml): 1% (44.2, 2.9), 6% (55.2, 8.3, $p<0.05$) and 20% (132.3, 19.6, $p<0.01$). No statistically significant differences were observed for the other proteins at 48h. H&E staining of fixed tissue showed no major morphological differences relating to oxygen levels.

Conclusions

First trimester serum PlGF levels are reduced in women who go on to develop pre-eclampsia so the finding that the placental production and/or release of PlGF is increased following exposure to hyperoxia is inconsistent with our original hypothesis. The data also suggest that first trimester placental tissue is broadly resistant to differences in oxygen exposure.

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Novel Expression Patterns of the Maspin Tumor Suppressor Protein in Preeclamptic and Egg Donor Placentas. Elizabeth S Taglauer,[1,2] Fusun Gundogan,[3] Kirby L Johnson,[1,2] Diana W Bianchi.[1,2] *[1]Mother Infant Research Institute, Tufts Medical Center, Boston, MA, USA; [2]Floating Hospital for Children, Boston, MA, USA; [3]Perinatal Pathology, Women and Infants Hospital, Providence, RI, USA.*

Maspin is a serine protease inhibitor with multiple functions including inhibition of cell invasion. This protein is expressed on human placental trophoblast cells and in vitro studies suggest it may modulate their migration. However, maspin has not been studied in preeclampsia (PE) or relative to the maternal-fetal immunological relationship, two clinical contexts that may influence trophoblast cell migration. Egg donation, which results in a fully allogeneic fetus, offers a unique opportunity to study the influence of an altered immunological environment during pregnancy. We thus examined maspin expression in placentas from either IVF or egg donor (ED) pregnancies with and without PE (IVF n=7; IVF-PE n=8; ED n=5; ED-PE n=7). All tissues were obtained from cesarean deliveries at 34-39 weeks with no significant differences in gestational age among the groups ($p= 0.087$). Using immunohistochemistry, maspin positive cells were identified throughout the placenta in all groups. Notably high concentrations were in scattered clusters at the chorionic plate. Co-staining with cytokeratin 7 confirmed these populations as extravillous trophoblast cells (EVT). We then counted the number of maspin positive cells per high power viewing field in various placental regions. Unexpectedly,

the greatest differences among groups were localized solely at the chorionic plate. Here, maspin positive cells were significantly decreased in IVF-PE vs IVF (p=0.005) and in ED vs IVF (p = 0.013). Other group comparisons and placental regions did not yield significant differences. These results suggest placental maspin expression may be influenced by preeclampsia and by the maternal-fetal immunological relationship. As maspin inhibits cell migration, its comparative downregulation on chorionic EVT may allow more invasion at the placental interface closest to the fetus. Further studies are required to determine the function of these localized cells, which could include nutrient delivery, endocrine, immunological, or other yet unidentified purposes. Overall our work identifies maspin expression in new clinical contexts and presents the chorionic plate as a potentially novel interface to examine alterations in trophoblast invasion among various pregnancy pathologies.

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TGFβ Regulation of TAZ-Par6 Interaction during Placental Development. Andrea Tagliaferro,[1] Tharini Sivasubramaniyam,[1] Isabella Caniggia.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Departments of Obstetrics/Gynaecology, Mount Sinai Hospital, Toronto, ON, Canada; [3]Physiology, University of Toronto, Toronto, ON, Canada.*

TGFβ signaling is vital in regulating trophoblast cell fate in pregnancy and its associated pathologies. Transcriptional co-activator with PDZ-binding motif (TAZ) has been recently demonstrated to contribute to TGFβ signaling by interacting with Smads. In addition, evidence has shown that Partionioning defective protein 6 (Par6), a key regulator of cell polarity, is central to the TGFβ Smad-independent signaling pathway. Like TAZ, Par6 also contains a PDZ motif. We hypothesize that TAZ interacts with Par6 via its PDZ motif thereby regulating trophoblast cell differentiation via TGFβ during placentation. Expression of TAZ and Par6 were examined in placental lysates throughout gestation by immunoblotting and dual-labelled immunofluorescence staining (IF) in placental sections. To establish TAZ-Par6 interaction, Flag-tagged wild-type TAZ, TAZ-PDZ deletion mutant and T7-tagged Par6 wild-type constructs were transiently transfected in JEG3 choriocarcinoma cells, followed by Flag immunoprecipitation (IP) and Par6 Western blot analysis (WB). Expression levels of TAZ, Par6, pSMAD2, and Rho-A were examined by WB in JEG3 cells in the presence and absence of TGFβ1/TGFβ3. Subsequently, TAZ-Par6 interaction was assessed by IP analysis in placental lysates across development and in JEG3 cells cultured with and without TGFβ1/3. To establish a role for TAZ and Par6 in trophoblast migration, we performed wound-healing assay using JEG3 cells followed by IF analysis. During placental development TAZ and Par6 protein expression and interaction peaked at 8-12 wks of gestation. Early on, TAZ and Par6 co-localized predominantly in the cytotrophoblast cells while with advancing gestation their spatial distribution shifted to syncytium. TAZ interacted with Par6 via its PDZ-binding motif in JEG3 cells and notably TGFβ3, but not TGFβ1, increased TAZ-Par6 interaction. Exposure of JEG3 cells to TGFβ1/3 increases pSmad2 and RhoA expression, but not TAZ, when compared to control untreated cells. During wound healing assay, TGFβ1/3 induced TAZ-Par6 association in nuclei and cytoplasm of migrating cells. Our data demonstrate a novel role for the Smad-independent signaling pathway during placental development, whereby TAZ-Par6 system induced by TGFβ3 may regulate trophoblast cells differentiation. (Supported by CIHR)

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Anti-Transglutaminase Antibodies Effects on Human Endometrial Angiogenesis. Chiara Tersigni,[1] Fiorella Di Nicuolo,[1] Roberta Castellani,[1] Giuseppe Maulucci,[2] Marco De Spirito,[2] Antonio Gasbarrini,[3] Giovanni Scambia,[1] Nicoletta Di Simone.[1] *[1]Department of Obstetrics and Gynecology, Università Cattolica Del Sacro Cuore, Rome, Italy; [2]Institute of Physics, Università Cattolica Del Sacro Cuore, Campobasso, Italy; [3]Department of Internal Medicine, Università Cattolica Del Sacro Cuore, Rome, Italy.*

Background

Celiac disease (CD) is an autoimmune enteropathy triggered in susceptible individuals by gluten. CD patients have circulating anti-transglutaminase antibodies (anti-TGA), mediators of intestinal and extra-intestinal manifestations of CD. The epidemiological association between maternal CD and increased frequency of obstetric failure is well established, although the pathogenic mechanisms are still poorly understood. Recently, we observed that anti-TGA IgG bind to trophoblast cell cultures causing an apoptotic damage and reducing cytotrophoblast invasiveness.

Since the placentation process requires a proper endometrial angiogenesis, our aim was to study the possible effect of anti-TGA on angiogenesis process.

Materials and Methods

IgG and IgA anti-TGA were purified from serum of CD patients on a gluten-containing diet through commercial kit. Endometrial tissues were collected from fertile women undergoing hysterectomy for fibroid uterus. Human endometrial endothelial cells (HEEC) were isolated and purified through tissue digestion, centrifugation, immune selection, and characterized by cytometry. In vitro binding assay on HEEC culture of IgG and IgA monoclonal and polyclonal anti-TGA was performed through ELISA and immunofluorescence staining. HEEC capability to form capillary-like tube structures in vitro was evaluated. In vivo angiogenesis in subcutaneous angioreactors treated with anti-TGA and implanted in mice was analyzed by spectrofluorimeter reader. Phalloidin-FITC staining of HEEC cytoskeleton F-actin fibres was performed and studied by inverted confocal microscope.

Results

We showed that anti-TGA: bind to HEEC in a dose and time dependent manner; significantly decrease the number and the total length of the tubules formed by HEEC; determine a disorganization of cytoskeleton F-actin fibres in HEEC; when inoculated in mice, cause a significant reduction of newly formed blood vessels.

Conclusions

We firstly demonstrated the anti-TGA-mediated impairment of human endometrial angiogenesis, providing an additional pathogenic mechanism of placental damage in CD able to explain its epidemiological association with adverse pregnancy outcomes.

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Kisspeptin Is Produced by Syncytiotrophoblast and Inhibits Cytotrophoblast Invasion. Ranjan Upadhyay,[1] Carla Fortique,[1] Audrey Hertenstein,[2] Rona S Carroll,[1] Ursula B Kaiser,[1] Wendy Kuohung.[1,2] *[1]Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Obstetrics and Gynecology, Boston University School of Medicine, Boston, MA, USA.*

Objective: Defective placental invasion has been implicated as the underlying pathology for preeclampsia and intrauterine growth restriction. The neuropeptide kisspeptin (encoded by the gene KISS1) and its receptor (KISS1R) are highly expressed by the placenta, but their roles in pregnancy remain unclear. However, kisspeptin has been previously shown to suppress metastasis in melanoma and breast cancers. This study investigates the expression of KISS1/KISS1R within the placenta and whether it plays a role in regulating placental invasion.

Methods: Second-trimester placentas were collected under an IRB-approved protocol and trophoblasts were isolated by enzymatic digestion, density gradient purification, and immunomagnetic depletion of leukocytes and fibroblasts. KISS1 and KISS1R expression in enriched fractions of syncytiotrophoblast (STB) and cytotrophoblast (CTB) were measured by RT-qPCR on total RNA extracted from cells immediately after isolation. Transwell assays were used to measure the effect of kisspeptin stimulation on CTB invasion. Cells were seeded on Matrigel-coated filters and allowed to invade towards decidua-conditioned media. Cells on the distal side of the filter were identified by cytokeratin-7 staining and counted in order to calculate invasion index.

Results: We detected consistently higher levels of KISS1 mRNA (25.8±9.2 fold difference) in STB relative to CTB within the same placentas (n=5). This confirms our previously reported finding that kisspeptin was detectable only in the STB layer in villous sections by immunofluorescent staining. By contrast, KISS1R mRNA levels were dramatically higher (18.1±3.4 fold difference) in the enriched CTB fractions. In transwell assays, treatment with 10^{-7} M kisspeptin suppressed CTB invasion to an average of 68% of untreated controls.

Conclusions: Our findings of KISS1 expression in the STB and KISS1R expression in the CTB suggest that kisspeptin acts through its cognate receptor expressed in cytotrophoblast to coordinate invasion into the uterine lining and maternal blood vessels. These findings support the hypothesis that the kisspeptin system may regulate placental development and invasion through a paracrine mechanism.

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Naturally Occuring Variation in Trophoblast Invasion as a Source of Novel Biomarkers. Marie van Dijk, Allerdien Visser, Janny Posthuma, Ankie Poutsma, Cees BM Oudejans. *Clinical Chemistry, VU University Medical Center, Amsterdam, Netherlands.*

During the first trimester of pregnancy fetal trophoblasts invade the maternal decidua, thereby remodelling the maternal spiral arteries. This process of trophoblast invasion is very similar to cancer cell invasion, with multiple

signaling pathways shared between the two. Previously, we identified *STOX1 Y153H* to be a susceptibility allele for preeclampsia, a disease characterized by reduced trophoblast invasion. Indeed, a significant reduction in outgrowth potential was found in placentas homozygous for the *Y153H* allele.

Here, we investigate if first trimester placental explants can be used to identify factors associated with changes in cellular invasion. DNA samples of first trimester placentas with known outgrowth potential were used to study the epigenetic status of the promoters of two matrix metalloproteinases (*MMP9*, *MMP2*) involved in both placentation and cancer, and eight tumor suppressor genes (*TP73*, *RASSF5*, *RASSF1*, *APC*, *DAB2IP*, *PRKCDBP*, *WT1*, *MORF4L1*) also known to show partial DNA methylation in first trimester placenta. From these genes, *PRKCDBP* and *MMP2* showed a significant linear regression where increase in methylation percentage coincided with an increase in invasion potential. To function as a non-invasive marker an (epigenetic) factor must be detectable in blood. Therefore, methylation of *PRKCDBP* and *MMP2* was tested in a 26 weeks pregnant plasma sample and compared to non-pregnant plasma samples. While *PRKCDBP* showed partial methylation in both pregnant and non-pregnant samples, the non-pregnant plasma samples were fully methylated for *MMP2*, while only partial methylation of *MMP2* was detected in the pregnant plasma sample. The unmethylated DNA in the pregnant plasma is most likely placental in origin. This suggests that the level of unmethylated DNA has the potential to be used as an invasion marker, where higher levels of unmethylated DNA indicate a lower invasion potential of trophoblasts.

These data provide evidence that human first trimester placental explants are an excellent *ex vivo* model system to identify factors and thus potential biomarkers associated with changes in cellular invasion, e.g. to detect pregnancy-related diseases or cancer metastasis. To identify novel biomarkers the next step is to correlate naturally occuring variation in invasion potential to changes in (epigenetic) factors by genome-wide approaches such as massively parallel sequencing.

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Regulation of Proliferation and Invasion of Trophoblast Cells by the A2B Adenosine Receptor. Natalia Darashchonak, Akin Sarisin, Brunhild Koepsell, Frauke M von Versen-Hoeynck. *Department of Obstetrics & Gynecology, Hannover Medical School, Hannover, Germany.*

Background: Shallow trophoblast invasion into the maternal spiral arteries that is leading to placental hypoxia is hypothesized to be involved in the pathophysiology of preeclampsia. Hypoxia is a potent stimulus for the release of adenosine. Women with preeclampsia show increased circulating concentrations of adenosine and placental expression of adenosine receptors is increased in preeclampsia.

Objective: We are working on the hypothesis that adenosine is involved in placental development and tested the hypothesis that the adenosine receptor subtype A2B is involved in trophoblast proliferation and invasion.

Methods: Human HTR-8/SVneo trophoblast cells and human uterine microvascular endothelial cells (HUtMVEC) were used to model cell proliferation and invasion in the presence or absence of adenosine receptor A2B agonist, antagonist or a combination of both under hypoxic (2% O2), normoxic (placenta at term, 8%) or standard tissue culture conditions (21% O2) after 48h of incubation. Data are shown as median ± SEM of fold changes compared to untreated control of the respective O2 concentration. Statistical analyses were performed with Wilcoxon-signed rank test. Probability values were considered significant at $p<0.05$. Results: The median proliferative capacity of trophoblast cells was increased after adenosine receptor A2B stimulation at 2% or 8% O2 (1.23 ± 0.04 or 1.14 ± 0.07, n=6). Trophoblast invasion into an endothelial monolayer was reduced by blocking of the receptor at 2%, 8% and 21% O2 (0.65 ± 0.05; 0.84 ± 0.06 and 0.67 ± 0.04, n=6-8). Conclusions: Our results suggest a regulatory role for adenosine receptor A2B in trophoblast proliferation and invasion and could contribute a protective role of A2B in placental development.

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Effect of Sildenafil Citrate within the Placenta: A Possible Mechanism for the Amelioration of Fetal Growth Restriction. Baylee M Webster,[1] Joanna L Stanley,[1,2] Christian F Rueda-Clausen,[1] Rajan Poudel,[1] Colin P Sibley,[2] Sandra T Davidge,[1] Philip N Baker.[1,2] *[1]Obstetrics/Gynecology and Physiology, University of Alberta, Edmonton, AB, Canada; [2]Maternal and Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Background: Pregnant mice deficient in the enzyme catechol-O-methyl transferase (COMT-/-) demonstrate fetal growth restriction (FGR) that is improved with Sildenafil treatment during pregnancy (1). This study focused on the potential mechanisms by which Sildenafil may mediate these improvements and tested the hypothesis that the drug acts through placental effects on oxidative stress or altered angiogenesis.

Methods: Pregnant COMT-/- and control (C57Bl6/J) mice received Sildenafil citrate (0.2 mg/mg in drinking water) or normal tap water from gestational day 12.5. At day 18.5, placentae were removed and sectioned in OCT or homogenized prior to Western blotting. Placental sections were stained with dihydroethidum to determine superoxide production and anti-nitrotyrosine antibodies to assess peroxynitrite production. Western blots determined expression of vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase (sFlt).

Results: Superoxide production (122 ± 19 vs. 109 ± 20 as % C57Bl6/J control), peroxynitrite production (45 ± 5 vs. 44 ± 5 mean fluorescent intensity), and VEGF expression (98 ± 9 VEGF:Actin as % of C57Bl6/J control) in placentae from COMT-/- mice were not significantly different from C57Bl/6J mice. Treatment with Sildenafil had no significant effect on superoxide production (96 ± 6 % C57Bl6/J control), peroxynitrite production (50 ± 4 mean fluorescent intensity) or VEGF expression (96 ± 13 VEGF:Actin as % C57Bl6/J control). sFlt expression was significantly reduced in placentae from COMT-/- mice compared to controls (41 ± 6 sFlt:Actin as % of C57Bl6/J control; $p<0.05$). Although there was a trend towards normalization with Sildenafil treatment, this did not reach statistical significance (70 ± 13 %; $p=0.19$).

Conclusion: Sildenafil does not mediate improvements in FGR through the oxidative stress pathway or by increasing the pro-angiogenic factor VEGF. Reduced levels of free sFlt in placentae from COMT-/- mice may indicate an increase in sFlt bound to VEGF, which would lead to a decrease in angiogenesis; it is not yet clear if Sildenafil acts by improving angiogenesis through this pathway.

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S-189

U6 and U2 snRNA Downregulation in Syncytiotrophoblast. Debra S Goldman-Wohl,[1] Caryn Greenfield,[1] Galia Skarzinski,[2] Ronit Haimov-Kochman,[1] Tal Imbar,[1] Ilana Ariel,[2] Simcha Yagel.[1] *[1]Center for Human Placenta Research, Department of Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [2]Pathology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*

Background: The syncytiotrophoblast is formed by fusion of the underlying cytotrophoblasts, and among its many tasks synthesizes hormones and steroids. Yet, studies of syncytiotrophoblast transcription are puzzling, demonstrating that most of the nuclei in the multinucleated syncytium are transcriptionally inactive. Since transcription is intimately connected to splicing we investigated spliceosome snRNAs, U6 and U2, in the placenta to further elucidate RNA activity in the sycytiotrophoblast.

Methods: RNA in situ hybridization was performed on human placental tissue sections and JEG-3 cells in culture, employing U6 and U2 LNA DIG labeled probes with fluorescent detection.

Results: snRNAs were downregulated in the syncytium throughout the course of normal pregnancy as well as in placental pathologies including: complete hydatidiform mole, persistent trophoblastic disease, preeclampsia and choriocarcinoma. U2 was downregulated temporally as the villi differentiated. In JEG-3 cells, U2 and U6 appeared in a diffusely distributed speckled nuclear pattern similar to interchromatin granule clusters (centers of storing and assembly of the splicing machinery necessary to accommodate active transcription sites) with nucleoli sparing and differential distribution during the cell cycle.

Conclusion: The characteristic nuclear speckle distribution in trophoblasts may indicate dynamic assembly of nuclear organelles during trophoblast differentiation. Furthermore, our finding that snRNA is downregulated in the syncytiotrophoblast adds another dimension to the enigma of a transcriptionally inactive but translationally active syncytium and suggests that the fusing cytotrophoblast may be the vehicle that contributes RNA to the syncytium.

S-190

Caspases in Hypoxia-Induced Cell Death in the Fetal Guinea Pig Brain. Qazi M Ashraf, Om P Mishra, Maria Delivoria-Papadopoulos. *Pediatrics, Drexel University College of Medicine and St. Christopher's Hospital for Children, Philadelphia, PA, USA.*

Background: Previously we have shown that hypoxia results in increased high affinity Ca^{++}-ATPase activity and Ca^{++}-influx in the guinea pig fetal brain at term. We have also shown that hypoxia results in increased expression of

proapoptotic proteins Bax and Bad in the term guinea pig fetal brain. It is known that increased ratio of Bax/Bcl-2 proteins leads to activation of the caspase-9 initiated cascade resulting in cell death.
Objective: The present study investigates the effect of hypoxia on the caspase-9 and caspase-3 initated cell death cascade in the cerebral cortex of guinea pig fetus at term and correlates it with DNA fragmentation.
Design/Methods: Pregnant guinea pigs at term (60 days) gestation were divided into normoxic (Nx, n=6) and hypoxic (Hx, n=6) groups. Hypoxia in the fetus was induced by exposing the pregnant guinea pigs to an FiO_2 of 0.07 for 60 min. Hypoxia was documented biochemically by ATP and phosphocreatine (PCr) levels. Cytosol was isolated and caspase-9 and caspase-3 activities and expression were determined. Brains were fixed with formalin. Paraffin tissue sections were processed to determine cell death and assessed histochemically by performing terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining.
Results: ATP (µmoles/g brain) was 4.66±0.33 in Nx, 2.39±0.34 in Hx. PCr (µmoles/g brain) was 3.50±0.74 in Nx and 1.56±0.66 in Hx. Expression ($ODxmm^2$) of active caspase-9 was 62.67±7.4 in Nx and 103.07±20.04 in Hx. Expression ($ODxmm^2$) of active caspase-3 was 70.34±10.84 in Nx and 135.70±27.84 in Hx. Caspase-9 activity (nmoles/mg protein/hr) was 0.6±0.2 in Nx and increased in the Hx group to 1.2±0.3. Caspase-3 activity (nmoles/mg protein/hr) was 22±1.3 in Nx and 32±6.3 in Hx. The hypoxic group had 82% TUNEL-positive cells, whereas the normoxic group had 18% ($p<0.05$). The results show increased activation of caspase-9 and caspase-3 and increased number of TUNEL positive cells in the cerebral cortical tissue of the hypoxic fetus.
Conclusion: Hypoxia results in activation of caspases as evidenced by increased activities and expression of caspase-9 and caspase-3 as well as increased numbers of TUNEL-positive cells. We propose that biochemical indices such as the activity and expression of caspase-9 and -3 and DNA fragmentation are reflective of programmed neuronal death and indicative of the predominance of caspase-9 pathway following hypoxia.

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Death by Cooperation: Mtd Interaction with Mcl1 and Its Killing Ability. Julia Garcia,[1] Isabella Caniggia.[1,2] *[1]Research, SLRI, Mount Sinai Hospital, Toronto, ON, Canada; [2]Obstetrics/Gynaecology and Physiology, SLRI, Mount Sinai Hospital, Toronto, ON, Canada.*
We have previously reported on the identification and characterization of MtdP, a spliced variant of Matador/Bcl-2 Ovarian Killer (Mtd/Bok), a member of the Bcl-2 family proteins. We showed that MtdP isoform is specific to the human placenta and its expression is elevated in placentae from pregnancies complicated by severe preeclampsia. In parallel, products of its binding partner, the anti-apoptotic protein Myeloid Cell Leukemia 1(Mcl1L), were found to be decreased in this pathology. Presently, the mechanisms by which MtdP induces apoptosis and its interactions with pro-survival Mcl1L and its pro-apoptotic protein products originated from either cleavage or splicing (Mcl1c157,McL1c127 and Mcl-1S) remains to be established. The development of doxycycline inducible cell lines expressing GFP-MtdP and selective deletions of its most hydrophobic domains including alpha helices 4, 5,6 and the trans-membrane domain TM, was used to examine the regulation of apoptotic processes as assessed by FACS analysis for AnnexinV and LDH analysis. Our results show that alpha helices 4 and 5 of MtdP are predominantly responsible for the induction of intrinsic apoptosis by this protein. Notably, using cross-linker BMH in preeclamptic tissue lysates and in HEK293 cell lines transiently transfected with Flag-MtdL and MtdP constructs, we found that both MtdL/P oligomerize generating high molecular weight products. Co-transfection experiments using Mtd/Mcl-1 isoforms in HEK293 cells revealed a cooperation between MtdL/P and Mcl1 pro-apoptotic protein products (Mcl1S, Mclc127,157) in triggering cell death as measured by Trypan blue exclusion. Moreover, immunoprecipitation experiments and immunofluorescence analysis showed a physical interaction of MtdP and pro-apoptotic Mcl products in the mitochondria and interestingly in organelles as ER and Golgi. These findings were extended to preeclamptic tissue where Mclc157 was found to be increased. Together, these results suggest that MtdP elicits its killing capacity through putative pore forming domains and oligomerization. In addition, Mtd isoforms co-operate with pro-apoptotic Mcl1 products in cell death. In particular Mclc157 may function as a scaffold protein to bring MtdL/P to the ER, allowing cell death by apoptosis-inducing in the ER beyond the mitochondria. (Supported by CIHR)

S-192

IL-17 Mediated Oxidative Stress Is an Important Stimulator of AT1-AA and Hypertension during Pregnancy. Pushpinder Dhillon, Jeremy Scott, Kedra Wallace, Judith Heath, Janae Moseley, James Martin, Gerd Wallukat, Ralf Dechend, Babbette LaMarca. *Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS, USA.*
BACKGROUND. Preeclampsia is vastly becoming associated with chronic immune activation. Recent studies show that preeclampsia is associated with interleukin-17 secreating TH17cells, an inflammatory cytokine strongly associated with autoimmune diseases. Severity of preeclampsia is strongly associated with levels of activating autoantibodies to the angiotensin II type I receptor. Reactive oxygen species (ROS) specifically placental oxidative stress, is thought to be one important pathophysiological mediator of preeclampsia.
OBJECTIVE. The objective of our study was to determine if chronic exposure to IL-17 during pregnancy increases blood pressure by stimulating oxidative stress and AT1-AAs.
METHODS. To answer this question four groups of rats were examined: NP (n=20), NP+ IL-17 (n=7), NP+ tempol (n=7) (a superoxide dismutase mimetic that scavenges ROS) and NP+IL-17+Tempol (n=12). On day 14 of gestation miniosmotic pumps infusing IL-17 (150 pg/day) were implanted while tempol was administered via drinking water. On day 18 mean arterial pressure (MAP) was recorded, plasma, urine, and tissue were collected for isolation of ROS, detected by chemiluminescent technique. Urinary isoprostane was measured by ELISA. AT1-AA were determined via cardiomyocyte assay of column purified serum IgG, chronotropic events are beats per minute.
RESULTS. MAP increased from 98+/-3 in NP to 123+/-3 mmHg in IL17 infused NP rats, Urinary isoprostane increased from 1029 +/- 1 to 3526 +/- 2 pg/mg/day, $p<0.05$. Placental ROS: 436 +/- 4 (n=4) in NP to 702+/- 5 (n=5) RLU/ml/min in IL-17 treated rats. AT1-AA increased from 0.41+/-0.05 bpm in NP rats (n=8) to 18.4 +/-1 in IL-17 infused pregnant rats (n=13). Administration of Tempol attenuated the blood pressure increase to IL-17. MAP was 102+/-5 in Tempol controls and 101+/-5 mmHg in NP+ IL-17 + tempol. Tempol attenuated placental ROS production to 459+/-5 (n=5) in IL17+Tempol, which was no different from Tempol controls, 474+/-5 (n=3) RLU/ml/min. Administration of Tempol blunted AT1-AA secretion to 7.3+/-0.6 bpm in NP+IL-17+Tempol treated rats (n=9).
CONCLUSION These data indicate that chronic IL-17 causes placental oxidative stress which serves as stimulus modulating AT1-AA production which plays an important role in mediating IL-17 induced hypertension during pregnancy.

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Activin A in Preeclampsia: Developing Novel Therapeutic Strategies. Rebecca Lim, Euan M Wallace. *The Ritchie Centre, Monash Institute of Medical Research, Clayton, Victoria, Australia.*
Background: Preeclampsia (PE) is a pregnancy specific disorder characterized by sudden onset of hypertension and proteinuria. There is increasing evidence to suggest that oxidative stress plays a profound role in the pathophysiology of preeclampsia. Aim: Determine role of Activin A in induction of oxidative stress. Hypothesis: Activin A is a key molecules involved in the pathophysiology of PE and is directly related to the oxidative stress. Results: Activin A directly increased production of reactive oxygen species (ROS) in HUVECs (***$p<0.001$). This was mitigated with the addition of follistatin 288 (FS288), SOD, tempol or apocynin. Activin A also increased 8-isoprostane levels in a dose-dependent manner (*$p<0.05$). Similarly, this effect was mitigated through the addition of FS288 and all antioxidants tested. A similar trend was observed when HUVECs were tested with 20% PE serum. Furthermore, we confirmed that administration of recombinant activin A into pregnant mice induced preeclampsia-like symptoms where we observed hypertension, proteinuria, elevated liver enzymes, premature birthing and fetal growth restriction. These symptoms were improved when the administers were given apocynin in their drinking water. Conclusion: We present a novel concept in treatment of preeclampsia. Limiting the bioavailability of activin A or reduction of oxidative stress is likely to augment widespread maternal endothelial dysfunction seen in preeclampsia.

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Altered Mechanisms of ASM Regulation and Processing in Preeclampsia. Megan Melland-Smith,[1,2,3] Martin Post,[4] Isabella Caniggia.[1,2,3] *[1]Research, Samuel Lunenfeld Research Institute; [2]Obstetrics & Gynaecology, Mount Sinai Hospital; [3]Physiology, University of Toronto; [4]Pediatrics, Hospital for Sick Children, Toronto, Canada.*

Sphingolipids act as bioactive mediators in several pathophysiological processes by regulating cell fate. In particular, ceramides (CERs) are key effectors in pathways initiated by diverse stress stimuli. CER metabolism is tightly controlled by balancing its synthesis and breakdown via the action of specific enzymes. Acid SphingoMyelinase (ASM) causes sphingomyelin hydrolysis and subsequent CERs generation. ASM is synthesized as a precursor in the endoplasmic reticulum (ER) with 6 N-linked oligosaccharide chains which are essential for ASM trafficking to the lysosomes where it is activated. The objective of this study was to examine CERs and ASM expression, function and processing in placentae from preeclamptic (PE) and normotensive age-matched control (AMC) pregnancies. Protein and mRNA expression levels of ASM were assessed by Western Blot analysis and immunofluorescence (IF) in AMC and PE placentae. ASM glycosylation was analyzed using peptide-N4-aspargine amidase F (PNGaseF) and tunicamycin. Human villous explants and JEG-3 cells were kept at either 3% or 20% O2. Cells were also treated with C-16 ceramide and sodium nitroprusside (SNP: 2.5 and 5 mM). Markers of autophagy, including LC3B-II and lysosomal activity were assessed. Lipid analysis in normal and PE placental tissue and in SNP-treated cells was performed using high power liquid chromatography linked to mass spectometry (MS/MS). MS/MS revealed a significant increase in CERs levels in PE relative to PTC and this associated with an increased ASM precursor levels. Similarly, exposure of explants and JEG-3 cells to SNP increased CERs and both ASM and its precursor. Tunicamycin decreased SNP-induced ASM expression while increasing that of its precursor. De-glycosylation using PNGase F reduced ASM and its precursor in AMC and to a lesser extent in PE lysates. Furthermore, IF analysis showed ASM accumulation in the ER following SNP and low oxygen treatments in JEG3 cells suggesting that oxidative stress status halts ASM shuttling to the lysosomes. Moreover, C-16 ceramide treatment resulted in increased expression of LC3B-II and lysosomal activity. In conclusion, altered ASM expression and processing in PE, due to oxidative stress, is responsible for CERs accumulation that in turn may contribute to increased cell death typical of this disorder. (Supported by CIHR)

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Transcriptomic Analysis of Placental Explants Treated with an Antiphospholipid Antibody: Following the TRAIL to Preeclampsia? Priyadarshini Pantham,[1] Qi Chen,[1] Cristin G Print,[2,3] Larry W Chamley.[1] *[1]Department of Obstetrics and Gynaecology, The University of Auckland, Auckland, New Zealand; [2]Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand; [3]Bioinformatics Institute New Zealand, The University of Auckland, Auckland, New Zealand.*

Preeclampsia is a hypertensive disease of pregnancy, the symptoms of which are preceded by maternal endothelial dysfunction. Throughout normal pregnancy, placental trophoblasts that die via programmed cell-death are shed into the maternal blood and are cleared without any effect on maternal physiology. In preeclampsia, more trophoblasts may die by necrosis and their clearance could trigger endothelial dysfunction. Antiphospholipid autoantibodies (aPL) increase the necrotic death of trophoblasts and also augment the risk of developing preeclampsia nine-fold. In this study we began to investigate how aPL induce necrotic death in trophoblasts using a transcriptomic approach. Microarray analysis showed that 98 mRNAs were significantly different in aPL-treated placentae ($p_{LIMMA}<0.01$). Real-time RT-PCR of 7 mRNAs confirmed the trends observed in the microarrays. Analysis of molecular pathways and robust permutation analysis showed that seven mRNAs encoding regulators of apoptosis were enriched in aPL-treated placentae ($p<0.01$). Microarray data showed that TRAIL mRNA exhibited the change of the largest magnitude (3.3-fold reduction) in aPL-treated placental explants, which was confirmed by real-time RT-PCR ($p_{TTEST}=0.007$). Immunohistochemistry demonstrated that the levels of TRAIL protein may be reduced in the syncytiotrophoblast of aPL-treated placental explants. This work shows that a) Antiphospholipid antibodies cause changes in the cell-death regulatory machinery of placental explants, and b) TRAIL protein appears to be down-regulated in placental explants treated with aPL, potentially affecting the cell death process. This work provides insight into the molecular mechanisms controlling the death processes in trophoblasts in pregnancies affected by antiphospholipid antibodies, and reveals a potential molecular target, TRAIL, as a candidate for further investigation of the regulation of normal versus aberrant death mechanisms in placental trophoblasts.

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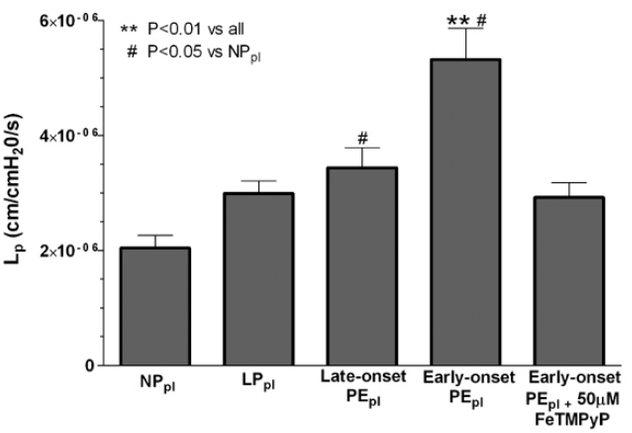
Peroxynitrite Decomposition Prevents Blood-Brain Barrier Permeability Induced by Early-Onset Preeclamptic Human Plasma. Malou P Schreurs,[1] Carl A Hubel,[2] Ira M Bernstein,[1] Marilyn J Cipolla.[1] *[1]Dept. of Neurology and Ob/Gyn, University of Vermont, USA; [2]Magee-Womens Research Institute, Dept. of Ob/Gyn, University of Pittsburgh, USA.*

Objective: Preeclampsia (PE) is a complex multisystem disorder with endothelial dysfunction at its center. Neurological complications resulting from blood-brain barrier (BBB) disruption do not occur in all PE women, suggesting different levels of severity, possibly reflected in the time of onset of PE. In addition, oxidative stress, particularly peroxynitrite ($ONOO^-$), is thought to be a key element in the development of endothelial dysfunction in PE. However, it is not known if the generation of $ONOO^-$ is also involved in disrupting the BBB. Here we determined the influence of plasma from normal pregnant, early-onset PE (<34 wks gestation) and late-onset PE (>34 wks) women on BBB permeability compared to plasma from nonpregnant women, and examined the influence of $ONOO^-$ generation on BBB permeability.

Methods: Plasma from nonpregnant (NP; n=9), normal pregnant (P; n=12), late-onset preeclamptic (LPE; n=10) and early-onset preeclamptic (EPE; n=5) women were pooled to minimize biological diversity. Cerebral veins from NP female SD rats were exposed intraluminally to 20% plasma in HEPES buffer from each group (n=6/group) for 3 hours. A separate set of veins was exposed to EPE plasma plus 50 μM of the $ONOO^-$ scavenger FeTMPyP. Hydraulic conductivity (L_p), a measure of BBB permeability, was compared.

Results: Both LPE and EPE plasma significantly increased BBB permeability (L_p, $cm/cmH_20/s$) compared to NP plasma ($p<0.05$) [Figure]. However, only EPE plasma significantly increased BBB permeability compared to all other groups, including LPE plasma ($p<0.01$ vs. all). FeTMPyP prevented the increase in BBB permeability induced by EPE plasma ($p<0.01$), suggesting $ONOO^-$ generation is an underlying mechanism of BBB disruption in early-onset PE.



Conclusion: Circulating factors present in EPE plasma increase BBB permeability through generation of $ONOO^-$, which may explain the increased risk of BBB disruption in EPE women. However, circulating factors responsible for $ONOO^-$ generation need to be determined.

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Premature Aging in Mice Exposed In-Utero to Nonionizing Radiation Emitted from Cellular Telephones. Tamir S Aldad, Hugh S Taylor. *OB/GYN, Yale School of Medicine, New Haven, CT, USA.*

Objective: The in-utero effects of cellphone radiofrequency exposure on development remain unknown. We evaluated the effect on memory in mice exposed in-utero to an 800-1900 Mhz device.

Methods: Thirty-three pregnant mice were exposed days 1-17 of gestation to 800-1900 Mhz cellular phones with a specific absorption rate of 1.6 W/kg. The phones were on an active call 24 hours/day and positioned at 15 cm. Forty pregnant controls were exposed to a deactivated phone. To evaluate behavior, twenty-six exposed offspring and twenty-six controls were given the standard object recognition memory test at three months of age and eighteen months of age. Three observers blinded to the treatments then scored the behavior. Corticosterone levels were measured by ELISA.

Results: The mean preference index determined by the object recognition memory test at three months of age was 80.5% in the control mice and 73.4% in the exposed group (control vs. exposed: $p < 0.05$), indicating an adverse effect on memory due to in utero cell phone use. The mean preference index decreased significantly in the control group to 66.7% at eighteen months (3 months vs 18 months: $p = 0.0017$). The mean preference index in the exposed group remained poor and was 71.4% at eighteen months, with the difference being statistically insignificant. Corticocosterone levels were identical between groups.
Conclusions: Control mice demonstrated a normal decline of memory with aging. Mice continuously exposed in-utero to cellular telephone radiation demonstrated premature aging characterized by poor memory, however the deficit did not progress over time. Exposure to cellular telephones in utero may lead to accelerated deterioration in memory that normally occurs during the aging process. In utero cellular telephone exposure may have an adverse impact on developmental programming of the brain.

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Prediction of Adverse Pregnancy Outcomes Using Urinary Metabolomic Profiling. Julia K Langer,[1] Yvonne EM Koot,[2] Nick S Macklon,[3] Phillip R Bennett,[4] Elizabeth Want,[1] Jan J Brosens,[5] Peter H Dixon.[4] *[1]Biomolecular Medicine, Imperial College London, London, United Kingdom; [2]Reproductive Medicine and Gynaecology, University Medical Centre Utrecht, Utrecht, Netherlands; [3]Developmental Origins of Adult Disease, University of Southampton, Southampton, United Kingdom; [4]Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom; [5]Division of Reproductive Health, University of Warwick, Coventry, United Kingdom.*
Background: Adverse pregnancy outcomes are common and distressing. Embryo implantation, limited to a few days of the cycle, is dependent upon embryo-endometrial cross-talk. Currently hCG is the most sensitive marker of implantation and patterns of urinary levels can give information on pregnancy outcome. Early pregnancy loss (EPL), defined as loss before clinical detection, can be attributed to impaired embryo selection by decidualizing endometrium. Decidualization is a phenomenon morphologically characterized by endometrial fibroblasts transforming into secretory epithelioid decidual cells.
Metabolomics offers an untargeted approach to the characterization of endogenous and exogenous metabolites in biological samples, enabling subtle changes due to diet, disease or therapeutic intervention to be determined. We investigated the hypothesis that urinary metabolic profiles reflect abnormal decidual phenotypes, and can predict adverse pregnancy outcomes at a early stage.
Methods: Daily early morning urine samples (n=191) of 2-3 subsequent cycles were obtained from 5 females, aged 27-32. 11 cycles were analysed; 6 from patients who suffered EPL, 3 from non-pregnant and 2 from pregnant patients. Global metabolic profiling was performed on each sample using ultra-performance liquid chromatography/ mass spectrometry (UPLC-MS) and metabolite identification via tandem mass spectrometry (MS/MS). Variability of the groups was assessed by multivariate statistics.
Results: UPLC-MS analysis revealed clear separation in urinary metabolic profiles between women who subsequently became pregnant and those that did not, as well as between those with EPL and pregnancy. Putative discriminatory metabolites identified by MS/MS included hippurate, as well as acyl carnitine, bile acid glucuronide, and steroid sulphate species.
Conclusions: Findings suggest a role for metabolomics approaches in urine screening for early prediction of adverse pregnancy outcome and for investigating decidual phenotypes.

S-199

Effects of Low Molecular Heparin within the Uteroplacental Unit. Siti Ismail,[1] Lucy Norris,[2] Lynne Kelly,[1] Shanthi Muttukrishna,[1] John Higgins.[1] *[1]Anu Research Centre, Dept of Obs & Gyn, Univ College Cork, Cork Univ Maternity Hosp., Cork, Ireland; [2]Coagulation Research Laboratory, Dept Obs & Gyn, Trinity Centre for Health Sciences, St James's Hosp., Dublin, Ireland.*
Background: LMWH is used widely for the prevention of placenta mediated pregnancy complications in thrombophilic patients. LMWH may be effective in altering local thrombin production in the uteroplacental compartment.
Aim: To determine the haemostatic effects of LMWH (tinzaparin) on the maternal systemic, uteroplacental and fetal circulation and on human haemostatic gene and antigen expression in placental tissue.
Method: Three women on antenatal LMWH prophylaxis (tinzaparin 75 IU/kg) due to moderate risk of VTE) undergoing caesarean section (CS) and a control group of 15 healthy pregnant women undergoing CS had venous blood sample taken from the antecubital fossa and uterine vein before delivery of placenta. Simultaneously, cord venous blood sample and placental biopsy was collected. Tissue factor pathway inhibitor (TFPI), thrombin antithrombin (TAT) and endogenous thrombin potential (ETP) assays were measured in plasma samples. Real-time PCR and ELISA were used to quantify mRNA and protein expression of TFPI, TFPI-2 and TF in placental tissue.
Results: TAT levels within uterine vein are significantly higher compared to maternal peripheral circulation in the control group ($P<0.01$). In the LMWH group, TAT was reduced compared with controls in the uterine vein ($P<0.02$). ETP levels between control and LMWH group in the peripheral, uterine or cord circulation did not differ significantly. Peak thrombin within cord circulation is significantly lower in the LMWH group compared with control group ($P<0.001$). TFPI within uterine circulation is reduced significantly in the LMWH group compared with control ($P<0.02$). A trend towards a down-regulation of placental TFPI mRNA and antigen expression was also found. Placental TF mRNA expression in LMWH group showed a non significant increase compared to control but this is not replicated in placental TF antigen expression.
Conclusion: TAT is reduced in uteroplacental circulation in thrombophilic women on LMWH prophylaxis. The potential for thrombin production in plasma as measured by ETP in uteroplacental circulation is not affected. LMWH effectively reduces *in vivo* thrombin production in the uteroplacental circulation of thrombophilic women without compromising the ability of the haemostatic system to staunch uteroplacental blood flow at delivery.

S-200

Lowering of Paternal a2V-ATPase Triggers Implantation Failure in the Female Mouse. Mukesh K Jaiswal,[1] Timothy M Mallers,[1] Gerard Chaouat,[2] Alice Gliman-Sachs,[1] Kenneth D Beaman.[1] *[1]Department of Microbiology and Immunology, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA; [2]INSERM U 782, Hospital Antoine Beclere Pavillon Jean Dalsacs, Clamart (Paris), France.*
Objective: The a2 isoform of vacuolar ATPase (a2V) is an immune-regulatory molecule and known to be a molecule responsible for survival of the fetal allograft. It is expressed in the matured sperm and considered as an important molecule for fertility. After fertilization, its expression starts with first zygotic cleavage and is maintained in the different stages of preimplantation embryos. Its deceases expression at the feto-maternal interphase results in poor pregnancy outcome. The objective of the present study is to evaluate the role of paternal a2V in pregnancy.
Methods: Five doses (5µg/animal) of anti-a2V-ATPase mouse IgG1 antibody (2C1) were injected *i.p.* in to the male Balb/c males on every alternate day. Control males were injected with mouse IgG1 isotype by the same manner. These males were used for mating with Balb/c females. The presence of vaginal plug was considered as day 1 of pregnancy. The status of pregnancy was checked on different days of pregnancy.
Results: The number of implantation sites was significantly lower (2.33 ± 1.2, $p\leq0.0013$) in the uterine horn recovered from female mated with male treated with anti-a2V than the female mated with the isotype control (9.17 ± 0.98) on day 7 of pregnancy. The antibody against a2V injected into the male exerts significant detrimental effect on the development of preimplantation embryos.
Discussion: The lowering of paternal a2V by injecting anti-a2V induces ~ 75% implantation failure. These results suggest that a2V plays a decisive role in male infertility, which is reflected by a poor pregnancy outcome in the females. These data also suggest that often male infertility may be interpreted as female infertility.

S-201

Oocyte Maturation: Biologic Role of Sterol Intermediates. Aaron Javitt,[1] Megan Smith,[1] John J Zhang,[2] James Grifo,[1] Mortimer Levitz,[1] Norman B Javitt.[1] *[1]Obstetrics & Gynecology, Medicine, New York University School of Medicine, New York, NY, USA; [2]New Hope Fertility Center, New York, NY, USA.*
The accumulation of sterol intermediates in the metabolic pathway from lanosterol to cholesterol during oocyte maturation is a unique event that is not known to occur in other tissues and implies biologic role(s) beyond that related to meosis activation (MA). Further insights on the success rate of IVF may be obtained by monitoring the patterns that occur in ovarian follicular fluid (OFF). We are analyzing sterol extracts of 50 µl aliquots of human OFF, before and after saponification, obtained from both natural and gonotrophin-induced cycles, by reverse phase HPLC-MS utilizing an APCI detector. Gradient elution resolves zymosterol (rt= 6.72 min), desmosterol (rt=7.10 min), ff-mas (rt= 7.75 min) and t-mas (rt=8.21 min) and other sterol intermediates without the need for preliminaryseparations or derivative formation that can

distort quantitative relationships. Surprisingly, desmosterol (confirmed by NMR analysis) not known to have MAS activity, was a predominant sterol intermediate often exceeding zymosterol. Desmosterol to cholesterol ratios increased after saponification but did not exceed. ff-mas to cholesterol ratios. Values were similar between the gonadotrophin-induced group (n=16) and natural cycle group (n=16). Statistically valid data regarding relationships of the different sterol intermediates to succsessful outome (pregnancy) are in progress. Both progesterone and the 17-alpha-hydroxy metabolite were present in all samples. In consonance with in vitro studies, these hormones can account for the variety of sterol intermediates that arise in OFF. It is of interest that the high proportion of desmosterol in OFF collected at the end of the cycle parallels the sterol intermediate composition of seminal fluid. This facile method permits monitoring of OFF at all stages of oocyte development, allowing for comprehensivevaluation of the role(s) of sterol intermediates in the maturation process.

S-202

Catecholamine-Induced Proliferation of Uterine Artery Endothelial Cells Is Mediated in Part Via De Novo Uterine Endothelial Synthesis of Catecholamines. Sheikh O Jobe, Orrianne R Morrison, Ronald R Magness. *Obstetrics and Gynecology, University of Wisconsin-Madison.*

Introduction: Recently we reported that catecholamines stimulate proliferation in uterine artery endothelial cells from the pregnant state (P-UAECs) via β-adrenergic receptors (ARs) and indeed play roles in angiogenesis regulation. However, the adrenomedullary or local source of these catecholamines remains unknown. Hypothesis: We hypothesized that catecholamine-induced proliferation of P-UAECs occurs via local de novo endothelial synthesis and secretion of catecholamines. Objectives: To evaluate: 1) expression of the catecholamine biosynthetic enzymes, tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), dopamine β-hydroxylase (DβH), and phenylethalomine-N-methyltransferase (PNMT) in P-UAECs and 2) if the catecholamine substrate L-Tyrosine can stimulate P-UAEC proliferation via β-ARs indicating de novo endothelial synthesis of catecholamines. Methods: Expression of TH, DDC, DβH, and PNMT was evaluated by Westerns. P-UAEC proliferation in response to (100nM-10mM) of L-Tyrosine, L-Arginine, L- Leucine, and L- Threonine was studied. Inhibition of β-ARs was performed using Propranolol (10μM) followed by treatments with L-Tyrosine, L-Arginine, L-Threonine, and L-Leucine. Proliferation was evaluated using BrdU incorporation. Results: We observed that P-UAECs express TH, DDC, DβH, and PNMT. The catecholamine substrate L-Tyrosine dose dependently increased proliferation in P-UAECs having a maximum proliferation of 2.6 ± 0.13 fold of the control ($P<0.05$) at the physiologic concentration of 10mM. The nitric oxide substrate L-Arginine did not induce proliferation in P-UAECs ($P > 0.05$); whereas, the nutritive amino acids L-threonine and L-Leucine stimulated proliferation in P-UAECs; responses were not dose-dependent and significantly less than L-Tyrosine responses. Propranolol inhibited proliferation of P-UAECs by 39% in response to L-Tyrosine, but had no effect L-arginine, L-Threonine, or L-Leucine responses. Conclusions: These results demonstrate for the first time that P-UAECs express all of the functional biosynthetic enzymes for de novo catecholamine synthesis. We also demonstrate that catecholamine-induced P-UAEC proliferation occurs partly via local de novo endothelial synthesis and secretion of catecholamines supporting the hypothesis of an autocrine/paracrine catecholamine loop in the regulation of angiogenesis during pregnancy. NIH HL49210, HD38843, HL87144, T32-HD041921

S-203

A Role for AKAP13 in Progesterone Receptor Action. Soledad Jorge,[1] Paul H Driggers,[1] Minnie Malik,[2] James H Segars.[1] *[1]Program in Adult and Reproductive Endocrinology, NICHD, NIH, Bethesda, MD, USA; [2]Department of OB/GYN, USUHS, Bethesda, MD, USA.*

Background: Fibroids are prevalent uterine tumors that are dependent on sex steroids for growth. Clinically, anti-progestins cause a reduction in fibroid size, but the mechanism of anti-progestin action is unclear. The Rho-GEF proto-oncoprotein AKAP13 is overexpressed in fibroids. AKAP13 has been shown to activate estrogen and glucocorticoid receptors, and bind these receptors via a LXXLL nuclear receptor-binding motif, but it is not known whether AKAP13 also affects progesterone receptor activation. The objective of this study was to determine whether AKAP13 affected ligand-dependent gene activation by progesterone receptor B (PR-B).

Methods: COS-7 cells were examined because they do not express endogenous AKAP13 or PR-B. Cells were transfected with a progesterone-responsive luciferase reporter (MMTV-tk-luc) or a basal promoter (tk-luc), as well as with expression vectors for PR-B and full-length AKAP13. Transfected cells were treated with progesterone (40nM P_4) or vehicle. After 24 hours, cells were lysed and assayed for luciferase activity. To further explore potential signaling pathways, cells were also treated with a p38 MAPK inhibitor (SB202190) or a MEK 1 and 2 inhibitor (UO126).

Results: Transfection of PR-B resulted in 5-fold ligand-dependent activation of the MMTV-luciferase reporter. Co-transfection of AKAP13 further enhanced P_4-dependent, reporter-specific activation by PR-B by 3 fold over ligand-dependent activation in the absence of AKAP13 ($p < 0.001$). In the absence of P_4, AKAP13 did not stimulate PR-B activation of the MMTV reporter. AKAP13 also had no effect on the basal-promoter, tk-luc, in either the presence or absence of P_4. Inhibition of p38 MAPK function did not affect PR-B activation by AKAP13, but inhibition of MEK 1 and 2 appeared to reduce AKAP13 activation of PR-B.

Conclusion: This is the first report that AKAP13 augments gene activation by PR-B in a ligand-dependent manner. Experiments are currently underway to further elucidate the specific signaling pathways involved and to test for an effect in cells that endogenously express AKAP13.

Support provided by the Clinical Research Training Program, a public-private partnership between NIH and Pfizer, and ZO1-HD-008737-10.

S-204

The Role of Soluble N-ethylmaleimide-Sensitive Factor Attachment Protein Receptors (SNAREs) in Physiologic and Pathologic TF Transport in the Endometrium. Graciela Krikun. *Ob/Gyn & Rep. Sci., Yale University, SOM, New Haven, CT, USA.*

Introduction: The switch from a non-receptive to a receptive endometrium is largely due to the action of progesterone which induces several molecules, among them, tissue factor (**TF**). Based on its structure and function it has been assumed that TF must be present on the cellular membrane. We now demonstrate that the localization of TF in human endometrial stromal cells (**HESCs**) is largely cytoplasmic in the secretory phase and becomes trans-membranal throughout pregnancy. Importantly, we show that in the absence of progestin, microbial components induce TF transport to the cell-membrane which may lead to dys-synchronization of the menstrual cycle, endometrial damage and infertility. Molecule transport is associated with SNARE complex-activation. We now show that SNAREs appear to be central in the patho-physiologic transport of TF in HESCs rendering SNAREs ideal targets for adverse clinical outcomes.

Methods: Confluent HESCs were treated with estradiol ($E2,10^{-8}$ M) or E2+progestin ($P,10^{-7}$ M). To simulate microbial infection, cells were treated +/- lipopolysaccharide (LPS) or the viral mimetic poly: [IC]. TF and SNARE analysis was conducted by IF, western blot analysis, ELISA or QRT-PCR.

Results: TF was present in the cytoplasm of HESCs treated with E2+P but absent with E2-only Tx. In the latter, the majority of TF was localized to the Golgi apparatus and the perinuclear envelope. Under pathological conditions in which a LPS or poly: [IC] were added, TF was highly expressed in the cell membrane. QRT-PCR and western blot analysis demonstrated that SNAREs were present in HESCs. LPS or poly: [IC] Tx. showed that TF was induced and localized to the HESC membrane and further co-localized with a target SNARE.

Discussion: SNAREs are essential in the docking and the subsequent fusion of diverse vesicle-mediated transport events. We now demonstrate that mRNA and protein for SNAREs are expressed HESCs. Co-localization of TF with the SNARE called membrin was demonstrated by IF after cells were treated with microbial agents. We propose a physiologic (progesterone- driven) or a pathologic (microbial-driven) mechanism involving SNARE activation and transport of TF to the cell membrane. A detailed understanding of TF cell trafficking is critical to the development of clinical interventions to restore fertility in patients with endometrial inflammation and/or infection.

S-205

Reproductive Aging Is Associated with Decreased Mitochondrial DNA in Murine Oocytes. Vitaly A Kushnir,[1] Tomika Ludaway,[2] Rodney B Russ,[2] Earl J Fields,[2] Christopher A Koczor,[2] William Lewis.[2] *[1]Department of Gynecology & Obstetrics, Emory University School of Medicine, Atlanta, GA, USA; [2]Pathology, Emory University School of Medicine.*

Significance: A key mechanism of female reproductive aging involves accumulation of chromosomal defects in oocytes. Mitochondria control energy metabolism through the electron transport chain (ETC); they are abundant in oocytes. Defects in mitochondrial ETC deplete ATP and thus compromise

energy necessary for oocyte spindle formation, checkpoints, and chromosome alignment which may promote chromosomal defects. The role of mitochondrial abundance in this process is not yet understood.
Objectives: Experiments were designed to create a model of reproductive aging using inbred C57BL6J mice and testing whether mtDNA depletion in oocytes is associated with reproductive aging and sub-fertility.
Methods: We operationally defined "young" (<100 days old) and "old" (≥300 days old) female C57BL6J mice. Each was bred with a "young" C57BL6J male. Reproductive parameters included time from pairing to conception, litter size, and live birth per dam at birth and at one and three postnatal weeks. In parallel, mtDNA abundance was quantified in individual oocytes from super-ovulated females of the groups by RT-PCR of mitochondrial cytochrome oxidase subunit 1 as the gene target.
Results: "Old" females exhibited prolonged time to conception and fewer surviving pups. Female age inversely correlated with number of live born pups per dam ($p<0.03$), pups surviving one week ($p<0.001$) and 3 weeks postnatally ($p<0.02$). mtDNA abundance in oocytes from "old" females was decreased 2.7-fold compared to those of "young" controls ($p<0.001$).
Discussion: These data establish a reproductive phenotype of aging in an inbred mouse model. Reminiscent of the human condition, fecundity is lower with advancing age while those that do get pregnant are less likely to deliver viable offspring. Data here demonstrate aging is associated with a significant decrease in mtDNA in oocytes. Interestingly, a threshold in mtDNA content appeared in "old" mice. The relationship between mtDNA content of oocytes and reproductive competence in aging needs further clarification.

S-206

A New Surgical Model for the Induction of Uterine Distension in the Rat. Maurizio Mandala,[1,2] Shannon Kostin,[1] George Osol.[1] [1]*Obstetrics and Gynecology and Reproductive Scinces, University of Vermont, Burlington, VT, USA;* [2]*Cell Biology, University of Calabria, Arcavacata di Rende (CS), Calabria, Italy.*
Background: The growth of the uterine circulation during gestation is requisite for normal pregnancy outcome, and its abrogation is associated with preeclampsia, fetal growth restriction and placental underperfusion. The mechanisms that underlie remodeling are not well understood.
Objective: To develop a surgical technique for inducing progressive stretch of the uterus, such as occurs in gestation, to determine whether uterine stretch alone can induce vessel remodeling similar to that seen in gestation.
Methods: Two surgical approaches were developed. The first involved a one-time infusion of 0.4 ml of medical grade silicone into the lumen of one uterine horn; animals were sacrificed after three weeks to examine the uterus and its blood vessels. In the second (progressive) method, silicone was infused daily for 10 days using an indwelling, catheter exteriorized to the periscapular region. Intrauterine pressure was used as a feedback signal to guide and normalize the degree of distension. Uterine weight and volume, myometrial cell division (Ki67), and uterine main and resistance (radial) artery length and diameter were measured to provide indices of vascular and myometrial remodeling.
Results: In addition to producing an initial stretch, a single infusion of silicone stimulated the formation of an endometrial transudate that resulted in a progressive increase in uterine volume to 14-fold above control values after 3 wks (2.8 ± 0.35 ml; n= 9). Transudate volume was greatly reduced in the daily/progressive silicone infusion protocol, which resulted in a final uterine volume of 3.8 ± 0.15 ml (n= 3). Intrauterine pressures decreased to baseline over a 24h period, indicating significant accommodation of the expanded volume. Both procedures augmented rates of cell division in myometrial and vascular smooth muscle and resulted in significant axial and circumferential growth of the mesometrial vasculature.
Conclusion: Even in the absence of fetoplacental units, myometrial stretch induces uterine vascular expansive remodeling in a pattern consistent with that observed during pregnancy. This new surgical model may prove to be useful for understanding the mechanisms and identifying the signals by which myometrial stretch stimulates uterine vascular growth during gestation.
Supported by NIH HL79253 and HL73895

S-207

Does Smoking Significantly Alter the Endocannabinoid Levels in the Seminal Plasma of Men Attending for Infertility? Timothy H Marczylo,[1] Akwasi A Amoako,[1] Emma L Marczylo,[2] Justin C Konje.[1] [1]*Endocannabinoid Research Group, Reproductive Sciences Section, CSMM, University of Leicester, United Kingdom;* [2]*Systems Toxicology Group, MRC Toxicology Unit, Leicester, United Kingdom.*
Introduction: The endocannabinoids, anandamide (AEA), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are a group of bioactive lipid signalling molecules known to be present in human seminal plasma. AEA has been shown to play a key role in the regulation of sperm survival, motility, capacitation and the acrosome reaction. OEA and PEA influence the effects of AEA through their effects on FAAH the enzyme that synthesises AEA. Cigarette smoking is associated with decreased sperm count and motility. Consequently we wanted to investigate the effects of smoking on seminal plasma levels of AEA, OEA and PEA.
Methods: Seminal fluid was collected from 13 men (6 smokers and 7 non-smokers) with normal semen parameters after 2-5 days of sexual abstinence. AEA, OEA and PEA levels were quantified by UHPLC-ESI-MS/MS
Results: AEA, OEA and PEA levels were all elevated in seminal plasma isolated from smokers (0.22, 2.68 and 13.2nM, respectively) compared to non-smokers (0.06, 0.41, 8.62nM, respectively) but only AEA levels reached statistical ($P=0.017$) significance (t-test).
Conclusions: Smoking changes the endocannabinoid content of seminal plasma. Since these molecules are essential for sperm function, changes in their levels in seminal plasma induced by smoking may represent a potential mechanism through which smoking induces reproductive toxicity in men.

S-208

Effect of Lactic Acid on Vaginal EMMPRIN Production: Implication for Altered Vaginal Microbiota-Related Pathology. Hannah Mossop,[1] Iara M Linhares,[1] Xia Zhou,[2] Ann Marie Bongiovanni,[1] Larry Forney,[2] William J Ledger,[1] Steven S Witkin.[1] [1]*Department of Obstetrics and Gynecology, Weill Cornell Medical College, New York, NY;* [2]*Department of Biology, University of Idaho, Moscow, ID.*
Introduction EMMPRIN (extracellular matrix metalloproteinase-inducer) is a glycoprotein on cell membranes and in extracellular fluids. It induces metalloproteinases (MMPs) and promotes endometrium breakdown and regeneration during the menstrual cycle and rupture of fetal membranes at parturition. EMMPRIN is also an essential co-factor for monocarboxylate transporter (MCT) proteins responsible for lactic acid transport under anaerobic conditions such as in the vagina. We evaluated vaginal EMMPRIN levels in women with and without a lactic acid bacteria-dominated vaginal microbiota as well as the effect of lactic acid on EMMPRIN production by vaginal epithelial cells.
Methods Vk2/E6E7, a vaginal epithelial cell line, was cultured for 24 hr +/- 2.5 – 20 mM L-lactic acid, +/- 50 ng/ml lipopolysaccharide (LPS). Vaginal bacterial communities in 31 women were determined by pyrosequencing the amplified V1-V3 region of 16S rRNA genes. EMMPRINin vaginal secretions was quantitated by ELISA. Differences in EMMPRIN levels were analyzed by the Tukey-Kramer multiple comparisons test and Mann-Whitney test.
Results A mean (SD) of 11.4 (2.2) ng/ml EMMPRIN was endogenously released into the culture supernatant by VK2 cells. Addition of physiological concentrations of lactic acid resulted in a progressive decline of EMMPRIN release ($p = 0.0391$). The inhibitory effect of lactic acid was lost if LPS was also included in the incubation mixture. Vaginal EMMPRIN levels were a median of 3.2 ng/ml and 7.5 ng/ml in women with and without a lactic acid bacteria-dominated vaginal microbiota, respectively ($p=0.0088$).
Discussion EMMPRIN is endogenously produced by vaginal epithelial cells and is needed to regulate lactate accumulation within epithelial cells at this site. We postulate that the down-regulation of EMMPRIN release by lactic acid mediates the balance between physiological and destructive EMMPRIN-related functions in the vagina. In the absence of a lactic acid bacteria-dominated microbiota, where lactic acid levels are drastically reduced, elevated local EMMPRIN production may increase susceptibility to MMP-mediated tissue destruction in both pregnant and non-pregnant women. A similar situation may result from the presence of a gram negative bacterial infection.

S-209

Rhesus Macaques Are Physiologically Relevant Models for Assessing Novel Therapies for Heavy Menstrual Bleeding in Women. Lindsay A Ohm, Courtney J Hergert, Ov D Slayden. *Division of Reproductive & Developmental Science, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA.*

INTRODUCTION: Heavy menstrual bleeding (HMB) impairs the quality of life of many women who ultimately resort to uterine ablative surgery or hysterectomy to control the condition. Development of effective therapies for HMB will require in vivo testing for efficacy and safety. We validated the use of macaques as a model for quantifying menstrual blood loss (MBL). To demonstrate the physiological relevance of the macaque model, we treated the animals with two anti-fibrinolytic therapies, often indicated as first-line treatment for HMB in women.

METHODS: Animal studies were reviewed and approved by the ONPRC Institutional Animal Care and Use Committee. Artificial menstrual cycles were induced in ovariectomized rhesus macaques by treating the animals with Silastic implants that released estradiol (E_2) and progesterone (P). Normal 28-day cycles were created by treating animals with E_2 for 14 days and then E_2 plus P for 14 days. Extended 35-day cycles were created by treating animals with E_2 for 14 days and E_2 plus P for 21 days. In each case P withdrawal induced menstruation. To assess the anti-fibrinolytic approach we treated animals (n=8) with transexamic acid (TXA; 75 mg/kg/day) or ε-aminocaproic acid (EACA; 100 mg/kg/day) beginning on cycle day 0 for 5 days. Menstrual flow was collected by vaginal tampon. The tampons were dried, homogenized with 5% NaOH, and the alkaline haematin extract was quantified spectrophotometrically.

RESULTS: Mean (± SE) MBL over three control 28-day cycles was 7.9 ± 0.57 mL per menstruation. Extending the menstrual cycle to 35 days significantly increased MBL to 13.7 ± 2.03 mL per menstruation ($P<0.05$). Treatment of animals on 28-day cycles with TXA and EACA significantly reduced MBL to 5.9 ± 0.50 mL and 6.6 ± 0.56 mL per menstruation, respectively ($P<0.05$). Treatment of animals on 35-day cycles with TXA only reduced menses to 9.39 ± 1.45 mL ($P<0.07$).

CONCLUSION: Accurate MBL measurements can be obtained from rhesus macaques, and macaques like women, respond to TXA and EACA with reduced bleeding. However, the anti-fibrinolytic approach was more effective in animals with normal MBL than animals with heavy MBL. These results demonstrate the physiological relevance of rhesus macaques as an animal model for testing therapies to reduce menstrual bleeding in women.

Supported by NIH RR000163.

S-210

Investigation of Placental Proteins To Predict Threatened and Recurrent Miscarriage. Michaela Peer, Lynne Kelly, Keelin O'Donoghue, Shanti Muttukrishna. *Obstetrics and Gynaecology, Anu Research Centre, Cork University Maternity Hospital, University College Cork, Cork, Ireland.*

Miscarriage is the most common complication of pregnancy. Although the physical impact may be small, miscarriage often accounts for serious psychological consequences in mothers. Placental proteins, like inhibin A and activin A have been shown to be important for the establishment and maintainance of pregnancy. The objective of the study was to investigate if these proteins are altered in maternal circulation of patients with symptoms of threatened miscarriage or a history of recurrent miscarriage compared to normal pregnancy.

Pregnant women with a history of recurrent miscarriage (n=12), with symptoms of threatened miscarriage (n=16) and healthy ongoing singleton pregnancy (n=18) were recruited at 4-9 weeks gestation. At the time of recruitment all women answered a questionnaire and a venous blood sample was taken. Serum was separated and stored at -80oC for analysis. Maternal serum inhibin A and activin A were measured using specific two-site immunoassays in the laboratory. After 9 month follow up, pregnancy outcomes were recorded, along with details of maternal age, BMI, smoking status and antenatal treatment.

There was no significant difference in the levels of activin-A and inhibin A between the three groups of pregnant women. However, in patients with symptoms of threatened miscarriage, the levels of inhibin A were significantly lower in women with a subsequent miscarriage compared to gestation matched term live births ($p=0.019$). The levels of activin A were significantly lower in women with a high BMI (>25 kg/m2), compared to under-or normal weight women (BMI<25 kg/m2) ($p=0.001$). Further, the levels of inhibin A had showed a trend towards significance and were lower in women with a high BMI ($p=0.055$).

In summary, we showed that inhibin A could be predictive of a subsequent miscarriage in women with symptoms of threatened miscarriage. Further larger studies matching patients for variables such as BMI are needed to evaluate the significance of these results.

S-211

Nuclear Magnetic Resonance Analysis of Embryo Spent Culture Media from Human Trisomy 21, Monosomy 21 and Normal Day 3 Embryos Reveal Differences in Their Early Metabolism. Imma Sanchez Ribas,[1,2] Francisco Dominguez,[2] Leonor Puchades,[3] Marissa Riqueros,[1] Pablo Vime,[4] Agustin Ballesteros,[1] Carlos Simon.[2] *[1]Gynecology, IVI Barcelona, Barcelona, Spain; [2]Research, Fundacion IVI, Valencia, Spain; [3]Research, Centro de Investigación Príncipe Felipe, Valencia, Spain; [4]Biologist, IVI Sevilla, Sevilla, Spain.*

Background: Many studies have been focused on the identification of metabolomic markers that will allow a better embryo selection in IVF programs.

Objective: To identify chromosome 21 metabolic biomarkers using Nuclear Magnetic Resonance (NMR) analysis of embryo spent media from chromosomally normal, monosomic 21 and trisomic 21 human embryos previously diagnosed by FISH

Methods: Ninety-eight embryos from our PGD/PGS program were biopsed and chromosomally analyzed by conventional FISH with a single blastomere using probes for chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y. A total of 127 spent media were collected at day 3 from chromosomally normal embryos (n=39), trisomy 21 (n=35), monosomy 21 embryos (n=24) and matched control media without embryos (n=29). Metabolomic analysis was performed using high-resolution Nuclear magnetic resonance (NMR) spectroscopy. Peak Areas of differential spectra compounds were quantified and means between groups compared.

Results: The spectra comparison between the indicated groups revealed differences in the regions where the methyl groups of Leucine/Isoleucine resonate. According to these results, trisomy 21 embryo samples have a significant lower (about 60%; $p<0.05$) Leucine/Isoleucine levels compared to monosomy 21 embryo samples when student t-test mean comparison were applied.

Conclusions: This study suggests that non-invasive NMR metabolomic profile of spent media from day-3 embryos could be useful to detect aneuploidies of chromosome 21 prior to embryo transfer.

S-212

Canine Meiotic Sex Chromosome Inactivation in Combination with Complete Heterologous Synapsis. Sam Schoenmakers,[1] Frederica Federici,[2] Evelyne Wassenaar,[2] Godfried van der Heijden,[2] Joop Laven,[1] Anton Grootegoed,[2] Willy Baarends.[2] *[1]Obstetrics&Gynaecology, Erasmus MC; [2]Reproduction&Development, Erasmus MC.*

During meiotic prophase in mouse spermatogenesis, pairing problems between autosomal chromosomes result in meiotic silencing of unsynapsed chromatin (MSUC), causing transcriptional inactivation. However, if heterologous autosomal regions manage to synapse, a mechanism called synaptic adjustment, MSUC is avoided and transcription continues.

Mammalian heterologous sex chromosomes are always faced with pairing problems due to evolutionary physical and genetic divergence. Therefore, the sex chromosomes synapse only partially and are transcriptionally silenced through meiotic silencing of sex chromosomes (MSCI), a specialized form of MSUC. The earliest marker of MSCI on the so-called XY body known to be essential for silencing is γH2AX.

We investigated the behaviour of X and Y chromosomes during canine male meiotic prophase in order to study the possible variability in sex chromosome behaviour during meiotic prophase between mammalian species. This knowledge may help to identify critical components of the MSCI/MSUC pathway common among mammals.

In contrast to male mouse sex chromosomes, the canine XY pair was found to synapse extensively in the meiotic prophase. The canine X chromosome shows persistent foci of the homologous recombination repair protein RAD51, but with progression of the heterologous synapsis between X and Y during pachytene, the number of RAD51 foci decreases. At mid-late pachytene, all RAD51 foci and γH2AX have disappeared, whereas the mouse X chromosome maintains RAD51 foci throughout pachytene and γH2AX until late diplotene. We postulate that the achievement of heterologous synapsis within the sex chromosomes in canine pachytene spermatocytes may facilitate homologous recombination repair of persistent meiotic DSBs, probably using the sister chromatid as a template for repair, allowing a more rapid repair of meiotic

DSBs on the canine X. These data indicate that γH2AX is lost from the sex chromosomes when repair is completed, even when the X and Y desynapse again in late pachytene. Despite the loss of γH2AX, the presence of a high level of H3K9 trimethylation on the XY body in late canine spermatocytes indicates that once MSCI is trigged, it remains activated, even in the presence of extensive heterologous synapsis. These findings provides a novel insight in the link between asynapsis, meiotic DSB repair and MSCI.

S-213

Tissue Factor Dependent and Independent Thrombin Generation across Pregnancy. Kelley C McLean,[1] Saulius Butenas,[2] Ira Bernstein,[1] Kathleen Brummel-Ziedins.[2] *[1]Obstetrics, Gynecology and Reproductive Sciences, Fletcher Allen Healthcare/University of Vermont, Burlington, VT, USA; [2]Biochemistry, College of Medicine, University of Vermont, Colchester, VT, USA.*

Normal pregnancy results in a pro-thrombotic state, with increased risk of venous thromboembolism. Studies investigating the capacity of pregnant women to generate thrombin are limited. In this pilot study, we longitudinally evaluated thrombin generation in plasma from young, healthy nulligravid women (pre-conception, early pregnancy, late pregnancy, and post-pregnancy, n=20) using a modified thrombin generation assay and compared them to 10 control women at 2 time points. Contact pathway inhibited, re-calcified plasma (±inhibitory antibodies to factors(f) IXa, XIa, and tissue factor) was initiated with 5pM tissue factor and 20μM phospholipid in the presence of a fluorogenic substrate. In all samples, the maximum level (Max Level) and maximum rate (Peak Rate) of thrombin generation increased during pregnancy, with the highest maximum level (336±178nM vs. 81±41nM, p<0.001), and rate (146±77 RFU/s vs. 35±18 RFU/s, p<0.001) occurring in late pregnancy compared to pre-pregnancy, respectively. The area under the curve (AUC), or endogenous thrombin potential (ETP) also increased in pregnancy when compared to pre-pregnancy measurements (2410±543nM*min vs. 1162±446nM*min, p<.001). Subsequently, thrombin generated during late pregnancy decreased in the post-pregnancy samples (Max Level 101±81nM, p<0.001; Peak Rate 50±45 RFU/s, p<0.001; AUC 1392±718nM*min p<0.001), such that the post-pregnancy samples were not significantly different from the pre-pregnancy samples. Additionally, a majority of samples showed tissue factor-independent thrombin generation, which was blocked with the addition of either an inhibitory anti-fIXa or anti-fXIa antibody, with anti-tissue factor antibody having no effect. Our data provide evidence for an increase in tissue factor-dependent and independent thrombin generation with pregnancy progression, the latter an apparent consequence of the presence of circulating fIXa or fXIa in these women. Further, our findings support a post-pregnancy return to pre-pregnancy thrombin generation parameters.

S-214

Genomewide Association Study of Early Spontaneous Preterm Birth. Heping Zhang. *Biostatistics, Yale University, USA.*

Background: Despite decades of research, the etiology of spontaneous preterm birth (SPTB) is insufficiently understood to enable development of effective intervention strategies. Several genomewide association studies (GWAS) have recently identified SPTB genetic variants, yet replicating those findings across studies has proved difficult.

Aim: To identify common genetic variants associated with early SPTB.

Methods: Maternal DNA samples from 962 early SPTB (20 to < 34 weeks) and 962 controls (39 to < 42 weeks) were collected prospectively from participants of the Genomic and Proteomic Network for Preterm Birth Research. Genotyping was performed using Affymetrix SNP Array 6.0. Controls were recruited to match in 1:1 with cases by race/ethnicity (White, Hispanics, African Americans, and others), maternal age (<20, 20-29, 30-39, 40+), and parity. We used PLINK software for quality control and preliminary analysis. For the SNP quality control, we set thresholds for per-SNP missing rate at 0.05, per-individual missing rate at 0.05, minor allele frequency at 0.01, and Hardy-Weinberg disequilibrium p-value at 1.0E-5. We used logistic regression to include top SNPs simultaneously while controlling for matching variables.

Results: 804,917 SNPs met these thresholds. We selected the 20 SNPs using PLINK that are most significant individually, and the backward procedure in logistic regression selected a final set of the 9 SNPs on chromosomes 1, 3, 4, 5, 9, 10, 12, 15, and 17 whose p-values are less than 1.0E-4 in the regression model. The top 2 SNPs on chromosomes 3 and 12 have p-values of 3.0E-7 with odds ratios of 1.6 and 0.4, respectively. Based on the logistic regression, we defined a risk genetic variant as having at most one minor allele on Chromosome 9 locus and either one minor allele on Chromosome 12 locus or no minor allele on Chromosome 3 locus. The p-value for this genetic variant is 1.6E-11 after controlling for the matching variables and the other six top SNPs. This variant appears in 63.6% participants. The adjusted odds ratio is 2.22 (95% confidence interval 1.76 - 2.79).

Conclusion: None of the individual SNPs reached genomewide significance level of 1E-8; the combination of certain SNPs, however, did result in high genomewide significance. Our finding suggests that individual SNPs have low predictive power for SPTB, although combinations of SNPs may be predictive of subsequent SPTB.

Supported by U01 HD50062, HD50094, HD50088, HD50078, HD50080.

S-215

CD24: A Mediator That Distinguishes Damage-Associated Molecular Pattern from Pathogen-Associated Molecular Patterns Induced Inflammation in Normal Pregnancy, Labor, and Clinical Chorioamnionitis. Zeynep Alpay Savasan,[1,2] Tinnakorn Chaiworapongsa,[1,2] Nandor G Than,[1] Eleazar Soto,[1,2] Zhong Dong,[1] Sonia S Hassan,[1,2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*

Objective: Parturition at term is characterized by the presence of a physiologic inflammatory gene signature in myometrium and chorioamniotic membranes. Inflammation may be elicited by activation of Damage-Associated Molecular Patterns (DAMPs) or Pathogen-Associated Molecular Patterns (PAMPs), both of which interact with pattern recognition receptors. Distinction between DAMP and PAMP activation is established by CD24 which selectively represses DAMP, but not PAMP. The aim of this study was to determine if amniotic fluid soluble CD24 (sCD24) changes during pregnancy, pathologic (clinical chorioamnionitis) or physiologic (labor) inflammation at term.

Methods: Amniotic fluid (AF) samples were collected from patients in the following groups: 1) term not in labor (n=15); 2) term in labor (n=44); 3) term in labor with clinical chorioamnionitis (n=33); and 4) midtrimester (MT) (n=43). AF sCD24 concentrations were measured by ELISA.

Results: 1) sCD24 was detectable in 100% (135/135) of the samples; 2) the median AF sCD24 concentration was lower in patients at term in labor than those not in labor (not in labor median: 133.2 ng/mL, interquartile range (IQR): 97.7-147.9 ng/mL vs. in labor median: 84.2 ng/mL, IQR: 57.9-106.2 ng/mL; p=0.002); 3) term gestation had a higher median AF sCD24 concentration than MT (median: 133.2 ng/mL, IQR: 97.7-147.9 ng/mL vs. median: 28.2 ng/mL, IQR: 20.2-37.7 ng/mL ; p<0.001); and 4) the median AF sCD24 concentration was not different between patients with clinical chorioamnionitis and women at term in labor (median: 84.7 ng/mL, IQR: 69.9-108.1 ng/mL vs. median: 84.2 ng/mL, IQR: 57.9-106.2 ng/mL; p>0.05).

Conclusion: 1) sCD24 is a physiologic constituent of AF and its concentration in AF increases at term; and 2) physiologic (term labor), but not pathologic (clinical chorioamnionitis) inflammation is associated with a decrease in AF sCD24 concentration. We propose that sCD24 plays a role in the regulation of physiologic (sterile), but not pathologic inflammation.

S-216

Human Endometrial Stromal Pro-Inflammatory Cytokine Secretion Is Regulated by Ovarian Hormones and Aberrantly Elevated in Endometriosis. JC Chen, S Houshdaran, BA Johnson, K Vo, JC Irwin, LC Giudice. *Department of OBGYN and Reproductive Sciences, University of California, San Francisco.*

Background: Human endometrium actively produces cytokines during the menstrual cycle, which are involved in dynamic endometrial processes. This immune environment is influenced by ovarian hormones estradiol (E_2) and progesterone (P_4), and several cytokine-mediated pathways are up-regulated during the P_4-dominant secretory phase. Moreover, some endometrial disorders, including endometriosis, are associated with hormonal and immune dysregulation within the tissue. Herein we identified cytokines secreted by endometrial stromal fibroblasts (eSF), investigated their regulation by E_2 and P_4 and the differences in secretion between eSF from women with (Endo) vs. without (Non-endo) endometriosis.

Methods: Endometrial tissues were dissociated and eSF isolated by size fractionation and cultured to confluency at passage 3. eSF were then treated with 10nM E_2+1μM P_4 (E_2P_4) or ethanol vehicle for 15 days in DMEM/MCDB-105/2% FBS renewing media every 72h. Conditioned media were collected at days 3 and 15 of E_2P_4 treatment and analyzed by IGFBP1 ELISA to assess decidualization and by Luminex assay to measure secreted cytokines. T-test was used for data analysis, N=3.

Results: By 15 days of E_2P_4 treatment, Non-endo eSF had decidualized (200ng/ml IGFBP1) in contrast to Endo eSF or vehicle-treated controls. At day 15, Interleukin (IL)6 was higher in vehicle-treated Endo eSF vs. Non-endo eSF (403.1 vs. 201.8 pg/ml, $P<0.05$). E_2P_4 reduced IL6 levels in both Endo and Non-endo eSF (118.9 and 78.8 pg/ml respectively, $P<0.05$). IL8 was also higher in vehicle-treated Endo eSF vs. Non-endo eSF at day 15 (4730.9 vs 656.9 pg/ml, $P<0.05$). Contrary to IL6, E_2P_4 increased IL8 levels in both Endo and Non-endo eSF (6194.8 and 3558.9 pg/ml respectively, $P<0.05$). Similar patterns were observed at day 3 for both cytokines.
Conclusion: Our data indicate that E_2P_4 regulates pro-inflammatory cytokine secretion by eSF, suggesting a role for tissue cytokines in endometrial function. Increased IL8 secretion may contribute to increased HIV infection in women taking progestin-containing contraceptives. IL6 and IL8 are also significantly elevated in Endo eSF vs. Non-endo eSF, with or without E_2P_4, suggesting an altered endometrial cytokine milieu in this disease and identifying potential immune secretome markers of endometriosis.
Support: NIH U54HD055764-05, AI083050 (LCG)
*Equal contribution

S-217

Maternal-Fetal Interface Includes Memory T Helper Cells during Human Term Labor. Nardhy Gomez-Lopez,[1,5,6] Rodrigo Vega-Sanchez,[2] Guadalupe Estrada-Gutierrez,[3] Jorge Beltran-Montoya,[4] Felipe Vadillo-Ortega.[7] [1]*Research Direction, INPerIER;* [2]*Nutrition Research, INPerIER;* [3]*Infectology, INPerIER;* [4]*Toco-Surgical Unit, INPerIER;* [5]*OB/GYN, University of Alberta;* [6]*OB/GYN, University of Adelaide;* [7]*Biochemistry, Faculty of Medicine, UNAM.*
Objective: Normal human labor is characterized by infiltration of leukocytes into the maternal-fetal interface (choriodecidua). Current understanding is that these leukocytes may promote labor. We hypothesized that the maternal-fetal interface includes leukocytes that could promote and also regulate labor. Here, we characterized in detail the leukocyte phenotype of infiltrating leukocytes in the maternal-fetal interface before and during term, and during labor.
Methods: Fetal membranes (amnion and choriodecidua) were collected from three groups of women: 1) Preterm gestation non in labor (PTNL; 32.9 ± 2.4 gestational weeks (GWs); n=5), 2) Term gestation non in labor (TNL, 38.4 ± 1.1 GWs, n=7), and 3) Term gestation with active spontaneous labor (TL, 39.6 ± 0.31GWs, n=5). Histological slides and choriodecidual leukocytes were obtained from them. Leukocyte phenotype was determined by flow cytometry and immunofluorescence using monoclonal antibodies to identify: total leukocytes (CD45), monocytes (CD14), NK cells (CD56), B cells (CD19), T cells (CD3), T helper cells (CD4), cytotoxic T cells (CD8), naive leukocytes (CD45RA) and memory leukocytes (CD45RO).
Results: T cells and monocytes were higher at PTNL than at TNL ($p < 0.0001$ and 0.023). In addition, T cells were even higher at TL than at TNL ($p = 0.006$. In contrast, granulocytes were lower at TL than at PTNL and TNL ($p< 0.0001$ and 0.03). Within T cells, T helper cells were higher at TL and TNL than at PTNL ($p < 0.0001$ each). These T helper cells were localized into these tissues as memory cells, since they expressed CD45RO and their tissue density also increased gradually from PTNL to TL. In addition, we identified $CD3^+CD4^-CD8^-$ T cells (γδ T cells) in these tissues, which were higher at PTNL than at TNL and TL ($p < 0.0001$ each), and higher at TL than at TNL ($p = 0.05$). Statistical analyses used ANOVA and pos-hoc tests with significance at $p\leq0.05$.
Conclusion: Maternal-fetal interface is infiltrated with memory T cells including T helper cells and γδ T cells, which may regulate active labor at term of pregnancy.

S-218

Parturition Delay in Interleukin-6 Null Mutant Mice Is Not Linked with Gross Alterations in Leukocyte Subsets in the Maternal Periphery, Uterus or Decidual Tissues at Term Gestation. Nardhy Gomez-Lopez,[1,2] Camilla L Dorian,[1] Sarah Robertson.[1] [1]*Discipline of Obstetrics & Gynaecology, Robinson Institute, University of Adelaide, Adelaide, SA, Australia;* [2]*OB/GYN, University of Alberta, Edmonton, AB, Canada.*
Objective: Labor resembles an inflammatory response with infiltration of maternal peripheral leukocytes into uterine tissues. Interleukin-6 (IL6) plays a key role in controlling the timing of labor since in mice with a null mutation in the *Il6* gene (*Il6*-/- mice), delivery is delayed by 24 hrs. Since IL6 is linked with progression of the inflammatory response, we hypothesized that IL6 deficiency is associated with altered recruitment of leukocytes into the term gestational tissues.
Methods: Peripheral blood, uterine and decidual tissues were isolated from wild type C57Bl/6 (WT; n=6) and *Il6*-/- (n=5) mice at gestational day 19.5. Viable and resorbing implantation sites were counted. Uterine and decidual leukocytes were isolated from viable implantation sites after tissue dispersion. Leukocyte subsets were analysed by flow cytometry using mAbs: CD45 for total leukocytes, F4/80 for monocyte/macrophages, Ly-6G for neutrophils, CD11c for dendritic cells and CD3, CD4 and CD8 for T cells. Leukocyte subsets were quantified as a percentage of CD45+ cells after excluding non-viable ($DAPI^+$) cells. Statistical analysis was by ANOVA and T-test with significance defined as $p\leq0.05$.
Results: No differences were found in the relative proportions or total numbers of different leukocyte subsets in *Il6*-/- compared with WT mice. Within decidual and uterine tissues, monocyte/macrophages were more abundant that neutrophils and T cells. Neutrophils were relatively enriched in the decidua compared with uterus and blood ($p<0.05$). In contrast, T cells were less abundant in uterus and decidua than in blood ($p<0.007$). CD8+ (%CD3+) T cells were not different between compartments in WT mice; however in *Il6*-/- mice they were higher in the decidua than in the uterus ($p=0.024$). As reported previously, we found fewer viable implantation sites and elevated resorptions in *Il6*-/- compared with WT mice.
Conclusion: Interleukin-6 ablation impairs progression of labor however this not linked with overt differences in inflammatory leukocyte recruitment into the decidua and uterine tissues at term gestation. In ongoing studies we will examine the activation phenotype of leukocytes focusing on T cells which are known to be subject to IL6 influence.
Funding: NHMRC in Australia and MTPRF in Canada

S-219

Differences in the Adaption of the Innate Immune Response to Pregnancy in Rats with Type 1 Diabetes Versus Healthy Control Rats. Bart Groen,[1] Thera P Links,[2] Paul P van den Berg,[1] Marijke M Faas.[3] [1]*Obstetrics and Gynecology, University Medical Center Groningen, Netherlands;* [2]*Endocrinology, University Medical Center Groningen, Netherlands;* [3]*Pathology and Laboratory Medicine, University Medical Center Groningen, Netherlands.*
Introduction
The acceptance of the semi-allogeneic fetus during pregnancy is accompanied by adaptation in the maternal immune response. Aberrations in this could contribute to pregnancy complications, including miscarriage, preterm delivery and pre-eclampsia. Type 1 diabetes (T1D) is associated with an adverse pregnancy outcome, even despite stringent metabolic control. Therefore, other etiological factors may be involved. Since T1D is an autoimmune disease, the pregestational immune response of T1D women may differ from control women, possibly leading to aberrant pregnancy adaptations.
Objective
To assess adaptations of the innate immune response to pregnancy in a rat-model of T1D.
Methods
We included: 1) non-pregnant Biobreeding-diabetes prone (BBDP; an established model for T1D) rats; n=4, 2) pregnant BBDP rats; n=6, 3) non-pregnant Wistar rats (WR); n=6 and 4) pregnant WR; n=6. After establishing diabetes in BBDP rats (blood glucose >15 mmol/l), rats were treated with insulin pellets to maintain blood glucose <7.0 mmol/l. Pregnant rats (day10) and non-pregnant rats (follicular phase) were sacrificed to obtain peripheral blood samples.
Monocyte and NK cell numbers, subsets and activation status were determined by flow cytometry.
Results
The mean number of fetuses tended to be lower ($p=0.065$) in BBDP rats (8.0 ± 1.4) compared with WR (11.7 ± 1.9). One BBDP rat showed 1 resorption.
In pregnant WR, the percentage (of total leukocytes) of NK cells decreased compared with non-pregnant WR ($p=0.026$). The ratio of the percentages of non-classical/classical monocytes tended to increase ($p=0.065$) in pregnant WR compared with non-pregnant WR. Also monocytes showed an activated phenotype in pregnant WR (i.e. increased expression of MHC-II ($p=0.041$) and decreased expression of CD4 ($p=0.041$)) compared with non-pregnant WR. This is in contrast with BBDP rats, in which we found no differences in NK cells, ratio of monocyte subsets and activation status between non-pregnant and pregnant rats.
Conclusion
This study showed differences in the adaptation of the innate immune response to pregnancy between WR and BBDP rats. Such aberrant adaptations may be involved in the development of adverse pregnancy outcome in T1D.

S-220

Aberrant Pregnancy Adaptations in the Specific Immune Response in Type 1 Diabetic Rats. Bart Groen,[1] Thera P Links,[2] Paul P van den Berg,[1] Marijke M Faas.[3] *[1]Obstetrics and Gynecology, University Medical Center Groningen; [2]Endocrinology, University Medical Center Groningen; [3]Pathology and Laboratory Medicine, University Medical Center Groningen.*

Introduction

Acceptance of a semi-allogeneic fetus is dependent on development of tolerance of the specific immune system towards the fetus. Thus the maternal immune response adapts by, e.g., increasing numbers of regulatory T-cells (Treg) during normal pregnancy. Indeed, decreased numbers of Treg were found in pre-eclampsia and miscarriage.

Type 1 diabetes is an autoimmune disease and associated with a reduced number and function of Treg. Therefore, the pregestational adaptive immune response of women with T1D differs from control healthy women. This may result in aberrant immunological adaptation to pregnancy in T1D.

Objective

To assess adaptations of the specific immune response to pregnancy in a T1D rat-model.

Methods

Four groups of rats were included: 1) non-pregnant Biobreeding-diabetes prone (BBDP; an established model for T1D) rats; n=4, 2) pregnant BBDP rats; n=6, 3) non-pregnant Wistar rats (WR); n=6 and 4) pregnant WR; n=6. After establishing diabetes in BBDP rats (blood glucose >15 mmol), rats were treated with insulin pellets to maintain blood glucose <7.0 mmol. Pregnant (day10) and non-pregnant rats (follicular phase) were sacrificed to obtain peripheral blood. T-lymphocytes subsets were determined by flow cytometry.

Results

BBDP rats are lymphopenic due to a mutation in the *Gimap5* gene, leading to decreased percentages of lymphocytes (15.0 ± 2.7 vs. 70.8 ± 2.2; p=0.01) and T-lymphocytes (4.3 ± 2.7 vs. 61.5 ± 2.7; p=0.01) in non-pregnant BBDP rats vs. WR. The ratio of helper/cytotoxic T-cells did not differ between the strains. In WR, the percentage of effector T-cells (as % of helper cells (Teff)) increased from 5.7 ± 0.20 in non-pregnant WR to 7.5 ± 0.88 in pregnant WR (p=0.025). Also, the percentage of Treg increased from 3.1 ± 0.37 to 4.5 ± 0.66 (p=0.041) in this strain during pregnancy, although the ratio Teff/Treg did not differ (p=0.337).

In non-pregnant BBDP rats, percentages of Teff and Treg were 22.6 ± 2.90 and 11.6 ± 3.27 and increased as compared to WR (p=0.10). However, in contrast to WR, Teff and Treg did not increase during pregnancy.

Discussion

This study showed differences in the adaptation of the specific immune response to pregnancy in WR vs. BBDP rats. Especially, lack of expansion of Treg in BBDP rats during pregnancy may contribute to adverse pregnancy outcome in T1D.

S-221

Vitamin D Reduces Antiphospholipid Antibody-Mediated First Trimester Trophoblast IL-8 and sEndoglin Secretion. Stefan M Gysler,[1] Melissa J Mulla,[1] Jan J Brosens,[2] Larry W Chamley,[3] Anna K Sfakianaki,[1] Vikki M Abrahams.[1] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University; [2]Division of Reproductive Health, Clinical Sciences Research Laboratories, Warwick Medical School; [3]Department of Obstetrics and Gynecology, University of Auckland.*

Objective: Women with antiphospholipid syndrome (APS) are at risk of recurrent pregnancy loss (RPL) and pregnancy complications, like preeclampsia. Antiphospholipid antibodies (aPL) directly target the trophoblast by binding β2-glycoprotein I (β2GPI), which leads to altered cellular function. Pregnant women with aPL are treated with heparin alone, or in combination with aspirin. While this helps to prevent aPL-mediated RPL, it does not reduce the risk of preeclampsia, and exacerbates certain aPL-mediated effects on the trophoblast. Since vitamin D deficiency is common in patients with APS and is associated with pregnancy complications, like preeclampsia, the objective of this study was to determine the effects of vitamin D on trophoblast responses to aPL.

Methods: The first trimester trophoblast cell line, HTR8, was incubated with or without the mouse anti-human β2GPI mAb ID2 (20μg/ml) in the presence or absence of active vitamin D (1,25-dihydroxyvitamin D3; 10nM). Trophoblast secretion of pro-inflammatory IL-8, IL-1β, and IL-6; pro-angiogenic VEGF and PlGF; and anti-angiogenic sFlt-1 and sEndoglin were measured by ELISA. Trophoblast migration was analyzed using a two-chamber colorimetric assay.

Results: As previously reported, the anti-human β2GPI mAb significantly upregulated trophoblast secretion of IL-1β, IL-8, PlGF, VEGF, and sEndoglin, while secretion of IL-6 and sflt-1, and cell migration were significantly inhibited ($p<0.05$). Vitamin D alone had no effect on basal trophoblast cytokine or angiogenic factor secretion or on cell migration. The presence of vitamin D significantly reduced the ability of the aPL to upregulate trophoblast secretion of IL-8 by 24.2±11.6% and sEndoglin by 22.3±10.8% ($p<0.05$). Vitamin D had no significant effect on any other aPL-mediated effects on the trophoblast.

Conclusion: These findings demonstrate that vitamin D reduces the effects of aPL on first trimester trophoblast pro-inflammatory IL-8 and anti-angiogenic sEndoglin production. Thus, our data indicate a need for clinical studies to determine whether vitamin D supplementation is useful in the management of pregnant women with APS.

This work was funded by the American Heart Association.

S-222

The Immune Balance of Tumor Necrosis Factor (TNF) Superfamily in Fetal-Maternal Interface in Response to Pro-Inflammatory Stimuli. Min Li,[1] Chang-Ching Yeh,[1,2] Jillian Pecoriello,[1,3] Takugo Cho,[1,4] Salley Pels,[5] S Joseph Huang.[1] *[1]Dept. Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [2]Dept. Obstetrics and Gynecology, Taipei Veterans General Hosptial, Taipei, Taiwan; [3]Staples High School, Westport, CT, USA; [4]Northwestern University, Evanston, IL, USA; [5]Dept. Pediatrics, Yale University, New Haven, CT, USA.*

Context: The balance between the development of maternal immune tolerance to the semi-allogeneic fetus and the maintenance of defense against invading pathogens is critical during pregnancy. Excessive inflammation observed in decidua from adverse pregnancy outcomes, such as preeclampsia and miscarriage, suggests maternal immune maladaptation during gestation. TNF-like molecule 1A (TL1A), a member of tumor necrosis factor superfamily, can be induced in endothelial cells and immune cells in response to pro-inflammatory stimuli and leads to the induction of apoptosis in various cell types. Decoy receptor 3 (DcR3) is a soluble receptor capable of neutralizing the biological effects of TL1A, which prevents cells from apoptosis.

Objective: To examine the response of various cell types in the fetal-maternal interface to pro-inflammatory stimuli in the expression of TL1A and DcR3.

Methods: Human primary leukocyte-free 1st trimester decidual cells primed with estradiol (10^{-8} M) and medroxyprogesterone acetate (10^{-7} M), immortalized endometrial endothelial cells (iHEECs), and a 1st trimester extravillous trophoblast cell line (HTR-8/SVneo, HTR) were treated with or without 10 ng/ml IL-1β or TNF-α for 6h. The expression of TL1A and DcR3 were evaluated by quantitative reverse transcription-polymerase chain reaction.

Results: IL-1β and TNF-α up-regulated the expression of TL1A and DcR3 by 1st trimester decidual cells and iHEECs. The magnitude of TL1A induction was greater than that of DcR3. Only TNF-α increased TL1A expression in HTRs. However, neither IL-1β nor TNF-α increased the expression of DcR3 in HTRs.

Conclusions: Aberrant pro-inflammatory stimuli predominantly up-regulated TL1A expression in maternal and fetal cells in decidua. The induction of DcR3 to counteract this response was only observed in 1st trimester decidual cells and iHEECs with smaller magnitude. These findings suggest that the dysregulation of apoptosis-inducing TL1A and its antagonist, DcR3, in decidua in response to pro-inflammatory stimuli plays a potential role in immune maladaptation during pregnancy.

S-223

The Regulation of Macrophage Polarization by Colony-Stimulating Factors from Pro-Inflammatory Cytokine-Stimulated First Trimester Decidual Cells. Min Li,[1] Chang-Ching Yeh,[2] Zhen-Ming Wu,[3] Salley Pels,[4] S Joseph Huang.[1] *[1]Dept. of Ob/Gyn and Reproductive Sciences, Yale University, New Haven, CT, USA; [2]Department of Ob/Gyn, Taipei Veteran General Hospital, Taipei, Taiwan; [3]Dept. of Ob/Gyn, Renji Hospital, Shanghai Jiao Tong University, Shanghai, China; [4]Dept. of Pidiatrics, Yale University, New Haven, CT, USA.*

Objective: Macrophages (MØs) play important roles in the coordination of immune balance and vascular remolding in pregnancy. Decidual MØs in normal pregnancy are thought to be M2 dominant and the changes in their phenotype are proposed to be involved in the development of adverse pregnancy outcomes, such as preeclampsia. A decrease in M2 MØs was found in the decidua from preeclampsia with preterm delivery. Our previous studies revealed that: i) the preeclamptic decidua contained an excess of MØs and their differentiating factors, granulocyte-macrophage-colony-stimulating factor (GM-CSF) and M-CSF; ii) adverse pregnancy outcome-associated pro-inflammatory cytokines, interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), markedly enhanced the expression of GM-CSF and M-CSF in cultured leukocyte-free 1st

trimester decidual cells. Therefore, we hypothesize that 1st trimester decidual cell-secreted GM-CSF and M-CSF are responsible for the modulation of MØ polarization in response to pro-inflammatory stimuli.
Methods: Human peripheral $CD14^+$ monocytes (MOs) were differentiated into MØs with conditioned media (CM) from IL-1β- or TNF-α-stimulated 1st trimester decidual cells for 7d. As controls, MOs were differentiated ± GM-CSF ± LPS or ± M-CSF ± IL4, IL-1β or IL-10. The expression of polarization markers (CD80, CD86, CD163 and CD206) by MØs were evaluated by flow cytometric analysis.
Results: The expression of CD80, CD86, and CD206 in MØs was up-regulated by CM from IL-1β- or TNF-α-stimulated 1st trimester decidual cells ($p < 0.05$). However, the expression of CD163 was inhibited. Anti-GM-CSF neutralizing antibody suppressed the up-regulation of CD80, CD86, and CD206, whereas anti-M-CSF neutralizing antibody only inhibited the expression of CD80 and CD86. The treatment of anti-GM-CSF neutralizing antibody restored the expression of CD163.
Conclusion: These observations suggest that pro-inflammatory cytokine-stimulated 1st trimester decidual cells dictate MØ development toward M1 subtype. CSFs secreted by 1st trimester decidual cells, particularly GM-CSF, play potential role in this MØ polarization.

S-224

Positive C4d Immunostaining of Placental Cytotrophoblasts Supports Host-Versus-Graft Rejection in Villitis of Unknown Etiology. Meghan Gilroy, Erin Rudzinski, Terry Morgan. *Pathology and Obstetrics & Gynecology, Oregon Health & Science University.*
Background: Chronic villitis is observed in approximately 10% of placentas submitted for pathologic examination. It is characterized by lymphohistiocytic infiltration of the placental chorionic villi by predominantly maternal CD8 positive T-cells. Chronic villitis is associated with fetal growth restriction, preterm birth, and is most common in cases of recurrent pregnancy loss. The etiology is unknown, but accumulating evidence suggests the possibility of a host-versus-graft reaction, analogous to transplant rejection. Pathologists routinely screen for rejection in transplant biopsies by immunostaining for C4d, a classical complement component. Our objective was to test whether chronic villitis is more likely to immunostain for C4d than matched controls.
Methods: Chronic villitis was diagnosed in 82/1986 (4%) singleton placentas reviewed by one placental pathologist (TM) in our department from 2007-2011. We randomly selected 40 of these cases and 40 gestational age-matched controls for C4d immunostaining (Ventana Benchmark). Kidney transplant rejection sections served as a positive control. Positive C4d staining was defined as crisp, linear, circumferential staining of the syncytiotrophoblasts in at least one chorionic villous. Cases were independently scored by two placental pathologists (ER and TM) and discordant cases resolved by consensus before analysis. Cases were included for analysis if maternal age and parity were also available (cases n=37; controls n=39). Scoring reproducibility was evaluated by kappa statistic. Data were analyzed by Fisher's exact X^2 test and nonparametric Mann-Whitney U test.
Results: We observed very good scoring reproducibility between pathologists (kappa=0.79 [0.65-0.95]). Gestational age (36 weeks +/- 0.5) and maternal age (32 years +/- 1) were not significantly different between cases and controls. Positive C4d staining was strongly associated with chronic villitis (32/37, 86%) compared with controls (3/39, 7%) ($p<0.0001$). Staining was also more common in multiparous (33/62, 53%) than primiparous (2/14, 14%) women ($p=0.01$).
Conclusions: Positive C4d staining has been reported in cytotrophoblasts from patients with anti-phospholipid antibodies. The increased incidence of C4d staining in multiparous women and in placentas with chronic villitis supports the hypothesis that chronic villitis may also represent immune mediated rejection by the mother.

S-225

Defining the Chemokine Repertoire of the Mouse Female Reproductive Tract. Fiona M Menzies,[1,2] Robert JB Nibbs,[2] Scott M Nelson.[1] *[1]Life Course Nutrition & Health, College of Medicine, Veterinary & Health Sciences, University of Glasgow, United Kingdom; [2]Institute of Infection, Immunity & Inflammation, College of Medicine, Veterinary & Health Sciences, University of Glasgow, United Kingdom.*
Background: The mechanisms governing leukocyte recruitment to the female reproductive tract (FRT) play a critical role in protection from infection, remodelling during the estrus cycle, pregnancy and post-partum remodelling. Chemokines are key factors in driving tissue-specific leukocyte homing, yet little is known about their expression in the FRT. In this study, we have characterised the chemokine profile of distinct anatomical compartments of the mouse FRT at each estrus cycle stage.
Methods: Ovary, uterine horn, cervix and vagina were obtained from non-pregnant mice (n=20) during proestrus, estrus, metestrus and diestrus, and expression of 34 chemokines and 11 chemokine receptors were analysed using Taqman Low Density Arrays. Lung, skin, small intestine and colon were used as control tissues. Gene expression differences between tissues, and between estrus cycle stage were assessed by the Kruskal-Wallis test, followed by the Dunn's Multiple Comparison Test. $p<0.05$ was accepted as significant.
Results: The uterine horn exhibited expression of the largest number of chemokines, with CCL28 and XCL1 predominantly expressed. Uterine CCL28 expression was 20-fold higher than the ovaries ($p<0.05$), 50-fold higher than the cervix ($p<0.0001$) and 14-fold higher than the vagina ($p<0.05$). XCL1 expression was approximately 20-fold higher than the ovaries and cervix, and 30-fold higher than the vagina (all $p<0.0001$). Estrus cycle stage had minimal effects on chemokine expression, although CCL7 was reduced during estrus and metestrus in the ovary ($p<0.0269$), cervix ($p<0.0216$) and vagina ($p<0.015$), and CCR4 dropped during diestrus in the ovary ($p<0.0212$), uterine horn ($p<0.0291$) and vagina ($p<0.0051$). Analysis of leukocyte marker expression (F4/80, CD3, CD11c, CD19, CD56, MBP) suggests that the uterine horn is the principal home of most leukocyte subsets within the FRT, while the vagina exhibited low expression of CD56, a marker of NK cells.
Conclusion: This study provides a foundation for more detailed analyses of the role of chemokines in leukocyte trafficking to the FRT, and will aid studies aimed at defining FRT leukocyte function in female reproductive health.

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Missing Link between Stress and Infertility; Regulation of DAF by Stress Hormone. Stella Nowicki,[1] Rajbir Singh,[1] Tanu Rana,[2] Bogdan Nowicki.[2] *[1]Microbiology and Immunology, Meharry Medical College, Nashville, TN, USA; [2]Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*
PURPOSE: Decay Accelerating Factor (DAF) expression has been found to be reduced in patients with endometriosis and exhibiting luteal phase defects, both of which can lead to infertility. Multiple studies have shown association between stress and infertility. Although little is known regarding mechanism of DAF downregulation, we hypothesize that stress induced cortisol may dysregulate DAF. **METHODS:** ECC-1 is an epithelial cell line that maintains both estrogen receptors and androgen receptors which makes them ideal model system to study the effects of hormones in relation to both cancer and endometriosis. Cultured ECC1 cells were challenged with glucocorticoid for various time-periods. After treatment, RNA and protein was extracted and levels of DAF were determined by quantitative PCR and western blotting. Difference in the distribution of molecules at surface was determined by immunofluorescence staining. **RESULTS:** Cortisol treatment led to significant alterations in the levels of DAF observed both by quantitative PCR and western blotting. This leads to dysregulation of complement function and may be an important contributing factor to endometriosis. **CONCLUSION:** The present study implies that stress induced secretion of cortisol may lead to alteration in DAF levels predisposing the patients to endometriosis and infertility. This research was supported by NIH Grant # R01HDO55648-01 and by U54 RR026140 from National Center for Research Resources (NCRR).

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Increased Monocyte Activation in Pregnancies Complicated by Asthma. Annette Osei-Kumah,[1] Zarqa Saif,[1] Doreen Krumbiegel,[2] Ian Nicholson,[2] Randall Grose,[2] Nicolette A Hodyl,[1] Michael J Stark,[1] Heddy Zola,[2] Vicki L Clifton.[1] *[1]Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adealide, SA, Australia; [2]Leukocyte Biology Laboratory, Women's and Children's Health Research Institute, Adelaide, SA, Australia.*
Introduction: During pregnancy, we have shown that the asthma exacerbation rate is high, increasing the risk of an adverse neonatal outcome, including intrauterine growth restriction, preterm delivery and still birth. In a majority of cases exacerbations are un-resolvable with inhaled glucocorticoid treatment, suggesting pregnancy changes the asthma phenotype to a form that is non-responsive to asthma treatment. This may be related to maternal adaptations in immune pathways that occur with pregnancy. In normal pregnancy, maternal circulating leukocytes undergo modifications in cell concentration, phenotype and function over the course of pregnancy. However little is known about how this adaptation in pregnancy is influenced by the presence of maternal asthma. We aim to characterise leukocyte sub-populations and phenotypes in blood collected from pregnant non-asthmatic and asthmatic women.

Hypothesis: We propose that maternal asthma worsens during pregnancy due to altered leukocyte phenotypes, including increased monocyte CD14dimCD16+ subset, changes in markers of activation such as human leukocyte antigen (HLA)-DR and disturbances in T cell subsets particularly Tregs, Th1 and Th2.
Methods: Venous blood was collected from pregnant asthmatic subjects (n =10) and controls (n =10) at 12, 18 and 30 weeks gestation. Peripheral blood mononuclear cells (PMBCs) were isolated at all three time points. Multi-parameter flow cytometry analysis using appropriate antibody pairs was used to determine T cell and monocyte subsets.
Results: Our preliminary analysis demonstrated that there were no differences in T cell subtypes in control and asthma group as pregnancy progressed. However there were differences in monocyte subsets with differential expression of activation marker HLA-DR in the asthmatic group. Additionally, a subset of subjects had higher percentage of monocytes expressing HLA-DR. The expression of pro-inflammatory monocyte phenotype CD14dimCD16+ was also identified in some asthmatic subjects.
Conclusion: The differential expression of leukocyte subsets and activation states in pregnancies complicated by asthma may be part of the mechanism contributing to worsening asthma during pregnancy.

S-228

Decidual HLA-G+ T and Dendritic Cells with Potential Regulatory Effector Functions Are Overrepresented in Patients with Unexplained Spontaneous Abortion. Paola Panina,[1] Alessandra Mugione,[1] Giada Amodio,[2] Ana Maria Sanchez,[1] Edgardo Somigliana,[3] Massimo Candiani,[4] Paola Vigano,[1] Silvia Gregori.[2] *[1]Reproductive Sciences Lab, San Raffaele Scientific Institute, Milan, Italy; [2]TIGET, San Raffaele Scentific Institute, Milan, Italy; [3]Dept of Obstetrics and Gynecology, Fondazione Policlinico Cà Granda, Milan, Italy; [4]Department of Obstetrics and Gynecology, Università Vita-Salute HSR, Milan, Italy.*
At the maternal-fetal interface, the non-classical HLA-G antigen seems to be largely responsible for the reprogramming of local maternal immune response towards tolerance. A specialized subset of tolerogenic dendritic cells (DC) that secrete high amounts of IL-10 and express high levels of HLA-G and ILT4 are essential in the induction of regulatory T (Treg) cells in peripheral blood (1). The objective of the study is to investigate the frequency and function of tolerogenic HLA-G+ DC and T cells in decidua of patients with spontaneous abortion.
The decidual mononuclear cells from patients who experienced spontaneous abortion in the first trimester and from women undergoing elective abortion were collected and the proportion of tolerogenic DC-10 (CD14+CD16+CD11c+CD83+), expressing HLA-G and ILT4, of CD4+HLA-G+, CD8+HLA-G+ T cells and of Treg cells (CD25+ FOXP3+) were determined by flow cytometry.
A significantly higher proportion of tolerogenic DC-10 (2.8±0.9% vs. 0.9±0.7%, n=4, p=0.02) expressing HLA-G and ILT4 were found in decidua from spontaneous versus elective abortions. The proportion of CD4+ and CD8+ T cells in the decidua of spontaneous abortion (26.6±13.3% and 20.2±4.6%, n=4) was similar to that observed in the decidua from elective abortion (17.2±7.2% and 18.8±3.2%, n=4). However, higher proportions of CD4+HLA-G+ (37.2±16.2% vs. 13.6±3.5%, n=3) and CD8+HLA-G+ (21.1±9.4 vs. 3.0.±1.1%, n=3, p=0.028) T cells were observed in spontaneous versus elective abortions. This is the first report demonstrating the presence of HLA-G+ tolerogenic DC-10 and HLA-G+ T cells at the maternal-fetal interface. Our data suggest that HLA-G+ cells might accumulate in the decidua of spontaneous abortion in order to counteract the excessive inflammation associated with fetal rejection (2). We are currently investigating the role of these cells in terms of cytokine profiling, proliferation and ability to suppress effector T cells.
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2. Baricordi OR, et al. Inflamm Allergy Drug Targets. 2008 Jun;7:67.

S-229

Evidence That Maternal Floor Infarction Reflects an Abnormal Allogeneic Response (Maternal Anti-Fetal Rejection). Roberto Romero,[1] Amy Whitten,[1,2] Steven J Korzeniewski,[1,2] Nandor G Than,[1] Zhong Dong,[1] Sonia S Hassan,[1,2] Tinnakorn Chaiworapongsa.[1,2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI; [2]Dept Ob/Gyn, Wayne State University, Detroit, MI, USA.*
Objective: Maternal Floor Infarction (MFI) is a serious condition, often associated with fetal death and tends to recur in subsequent pregnancies. This study was conducted to determine if this complication of pregnancy could be the results of maternal anti-fetal rejection.
Methods: Pregnancies with placentas with MFI were identified (n=10). Controls consisted of women with uncomplicated pregnancies who delivered at term (n=180). Second trimester maternal plasma was analyzed for panel-reactive anti-HLA class I and class II antibodies. The prevalence of chronic chorioamnionitis (CC), villitis of unknown etiology (VUE) and plasma cell deciduitis (PD) was compared between cases and controls. Immunohistochemistry was performed to determine whether there was evidence of complement activation in the fetus (C4d deposition on umbilical cord vessels) (n=4). Specific maternal HLA antibody and fetal HLA-antigen status were also determined in paired specimens (n=6). Plasma CXCL-10 concentrations were determined in longitudinal samples of cases (n=28) and controls (n=759) by ELISA. Linear mixed models were used to test for differences in plasma CXCL-10 concentration.
Results: 1) Half of patients with MFI had fetal death; 2) The prevalence of VUE and PD was higher in MFI than in uncomplicated term deliveries (30% vs. 6.6%, p=0.04; 40% vs. 5.8%, p=0.004); 3) MFI was associated with a higher frequency of maternal anti-HLA class I seropositivity in the 2nd trimester than in the controls (80% vs. 36%, p=0.01); 4) Clear C4d deposition was observed on umbilical vein endothelium in all cases of MFI; 5) Specific maternal antibody against fetal HLA antigen was identified in all cases of MFI; 6) The mean maternal plasma concentration of CXCL-10 in MFI was higher than the controls (p<0.001).
Conclusion: MFI is associated with: 1) higher frequency of VUE or PD in the placenta; 2) presence of specific anti-HLA antibodies in maternal blood to fetal antigens; 3) evidence of antibody-mediated complement activation on umbilical vein endothelium; and 4) an elevation of a T-cell chemokine in maternal plasma. These findings indicate that a subset of patients with MFI have maternal anti-fetal rejection as a mechanism of disease. These observations have biological, diagnostic, prognostic, and therapeutic implications.

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The Th1 Versus Th2 Cytokine Milieu in Control and Preeclamptic Pregnancies: A Second Trimester Predictor of Preeclampsia? Mark Santillan,[1] Donna Santillan,[1] Wendy Hamilton,[1] William Rayburn,[2] Stephen Hunter,[1] Kimberly Leslie.[1] *[1]Obstetrics and Gynecology, University of Iowa Carver College of Medicine, Iowa City, IA, USA; [2]Obstetrics and Gynecology, University of New Mexico School of Medicine, Albuquerque, NM, USA.*
OBJECTIVE: Current data suggest that immune rejection of the feto-placental unit is an initiating molecular cause of preeclampsia. The cytotoxic, proinflammatory T helper cell 1 (Th1) response, important for the rejection of the feto-placental unit, prevails over the protective T helper cell 2 (Th2) response in preeclampsia. The objective of this study is to confirm this proinflammatory shift in preeclampsia and to determine if the specific second trimester Th1 or Th2 cytokine milieu predicts preeclampsia.
STUDY DESIGN: In this case-control study, gestational age-matched control and preeclamptic sera were obtained from the IRB approved University of New Mexico Maternal Bank. Effector proteins were measured with a multiplex protein array or ELISA. A 2 tail Student T test determined significant, differentially expressed cytokines. Receiver operator curves (ROCs) were generated to determine if the significant cytokines effectively predicted preeclampsia. $\alpha = 0.05$.
RESULTS: Th2 associated cytokines [TRAIL (p=0.009), IL 1 ra (p=0.001), IL 4 (p=0.001), and IL 13 (p=0.013)] in the second trimester are significantly lower in preeclamptics. Th1 associated cytokines [G-CSF (p=0.012) and IFN gamma (p=0.001)] are also significantly lower in preeclamptics. Antiangiogenic s-FLT1 was significantly elevated in preeclamptics (p=0.029). Conversely, proangiogenic IL 8 was significantly decreased in preeclamptics (p=0.016). By ROC analysis, s-FLT1 is the only analyte studied that can predict preeclampsia (AUC=0.75).
CONCLUSION: In concordance with present literature, we demonstrate that there is less of a protective Th2 response in preeclamptics. Yet, Th1 associated cytokines were also less in preeclamptics. Further molecular studies are needed to help determine if these second trimester findings are a cause of or a response to preeclampsia. Our second trimester maternal sera Th1 vs. Th2 cytokine evaluation is novel for the prediction of preeclampsia. Yet, we did not identify a reliable predictor cytokine. We confirmed the utility of s-FLT1 as a predictor protein for the development of preeclampsia. Further studies of this type in a more diverse population are needed to confirm our results.

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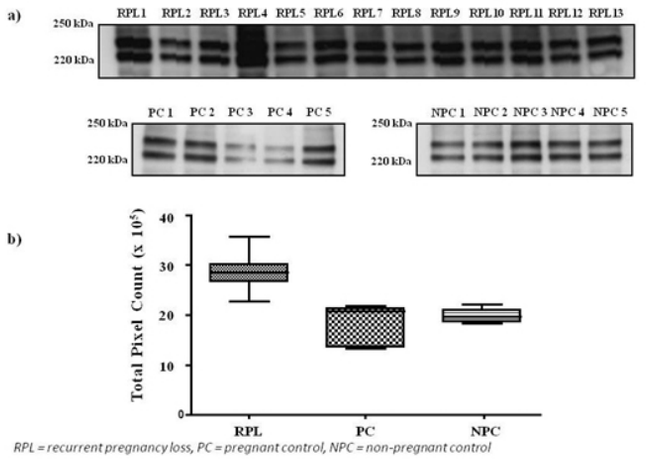
Alterations in Antibody Subclass Immune Reactivity to Trophoblast-Derived Fetal-Fibronectin and α-2-Macroglobulin in Women with Recurrent Pregnancy Loss. Rhiana D Saunders,[1] Steven T Nakajima,[1] Shesh N Rai,[2] Cicek Gercel-Taylor,[1] Douglas D Taylor.[1] *[1]Obstetrics, Gynecology and Women's Health, University of Louisville, Louisville, KY, USA; [2]Bioinformatics and Biostatistics, University of Louisville, Louisville, KY, USA.*

Background: Increasing evidence supports the involvement of complex antibody-mediated immunologic events at the decidua-trophoblast interface. Our objective is to define the humoral responses of pregnant women with a history of recurrent pregnancy loss (RPL) compared to gestation age-matched controls and non-pregnant controls in terms of trophoblast-derived antigens and IgG subclasses.

Methods: Immunoprecipitation and western immunoblotting was performed to characterize IgG subclass reactivity to Sw.71 trophoblast-derived fetal-fibronectin and α-2-macroglobulin, using serum obtained from first-trimester pregnant RPL subjects, gestation age-matched controls and non-pregnant controls.

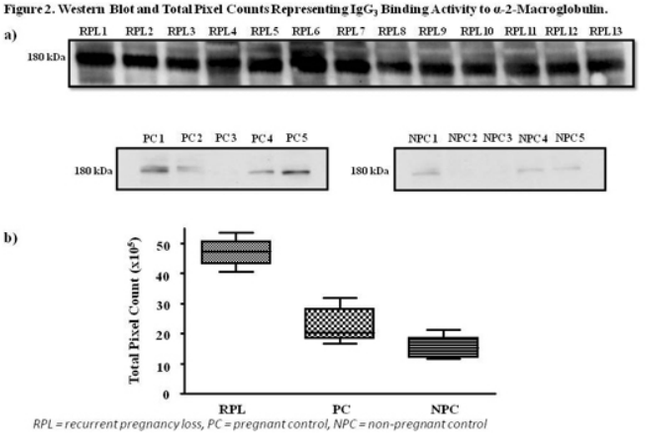
Results: The strength of maternal IgG_3 immunoaffinity for trophoblast-derived fetal-fibronectin and α-2-macroglobulin monomers are demonstrated in Figures 1 and 2, respectively,by western immunoblot pixel density.

Figure 1. Western Blot and Total Pixel Counts Representing IgG_3 Binding Activity to Fetal-Fibronectin.



RPL = recurrent pregnancy loss, PC = pregnant control, NPC = non-pregnant control

Figure 2. Western Blot and Total Pixel Counts Representing IgG_3 Binding Activity to α-2-Macroglobulin.



RPL = recurrent pregnancy loss, PC = pregnant control, NPC = non-pregnant control

Using a Generalized Linear Model, sera from women with a history of RPL exhibited increased IgG_3 immunoreactivity to trophoblast-derived fetal-fibronectin and α-2-macroglobulin compared to controls ($p < 0.0001$ and $p < 0.0001$).**Conclusions:** Complement-binding IgG_3 reactivity in women with RPL may play a significant role in aberrant immune-regulatory mechanisms in early pregnancy. Further investigations into the role of autoantibodies against trophoblast-derived proteins in implantation and pregnancy are warranted.

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Pregnancy Outcomes of Women with Failure To Retain Rubella Immunity. Christopher Schwartzenburg, Dzhamala Gilmandyar, Loralei Thornburg, David Hackney. *Ob/Gyn, Division of Maternal Fetal Medicine, The University of Rochester, Rochester, NY, USA.*

Objective: All pregnant women routinely undergo evaluation of their Rubella immunity secondary to its potential to wane over time and the possible negative impact during pregnancy. The reason for discordance in retention of immunity after vaccination is unknown, and may represent intrinsic differences in immunologic function between individuals. Several obstetrical complications, including pre-eclampsia and spontaneous preterm birth, are also hypothesized to involve immune system activation. Therefore, we sought to explore clinical variables associated with the loss of rubella immunity after an initial pregnancy and to determine if these changes were associated with increased odds of obstetrical complications.

Methods: This nested case-control study was performed within the cohort of patients delivering at a tertiary care hospital between 1/1/2005 and 12/31/2010. Subjects with chronic hypertension, autoimmune disease, pre-gestational diabetes, renal disease, immunosuppression, multifetal gestations, and chronic steroid use were excluded. In order to exclude subjects who may not have previously been vaccinated, women born outside of the United States and Canada, and those without a prior delivery were excluded. Cases of diminished immunity were defined as subjects whose rubella antibody titers were equivocal or non-immune. Controls were selected randomly from those rubella immune patients who otherwise met the inclusion criteria. T-test, Mann-Whitney U, Fisher's Exact test and logistic regression were used where appropriate.

Results: 285 cases and 285 control subjects were selected. Subjects with diminished immunity to rubella were more likely to have public insurance ($p=0.001$), and higher gravidity ($p= 0.003$). There was also a trend toward increased tobacco use among those with diminished immunity ($p=0.06$). There were no significant differences in race or age. Diminished rubella immunity was not associated with any adverse obstetrical outcomes, including preterm birth and pre-eclampsia, either in univariate analysis or when adjusted for potential confounders.

Conclusions: The diminution of rubella immunity after an initial pregnancy is associated with higher gravidity, tobacco use and public insurance, but does not appear to be independently associated with adverse obstetrical outcomes, including preterm birth and pre-eclampsia.

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Amelioration of Arthritis by Pregnancy in SKG Mice Correspond with Alterations in SAA3 Levels. Adrianne L Stefanski,[1,2] Laura A Shaw,[3] Lisa K Peterson,[3] Shimon Sakaguchi,[4,5] Virginia D Winn,[1] Leonard L Dragone.[3,6,7,8] *[1]Obstetrics and Gynecology, University of Colorado-AMC, Aurora, CO; [2]Graduate Program in Reproductive Sciences, University of Colorado-AMC, Aurora, CO; [3]Pediatrics, National Jewish Health, Denver, CO; [4]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; [5]WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan; [6]Integrated Department of Immunology, National Jewish Health, Denver, CO; [7]Pediatrics, Colorado Children's Hospital, Aurora, CO; [8]Pediatrics, University of Colorado-AMC, Aurora, CO.*

Objectives: Pregnancy leads to rheumatoid arthritis remission in humans. However, the immunologic mechanism that accounts for remission is not fully understood. Using the SKG mouse model of inflammatory arthritis, the objectives of this study were to 1) determine the impact of pregnancy on the maternal peripheral blood mononuclear cell (PBMC) transcriptome of arthritic SKG mice and 2) identify a biomarker associated with remission.

Methods: Cohorts of zymosan-treated pregnant SKG mice and controls were monitored for arthritis progression. Microarray analysis evaluated alterations in gene expression in maternal PBMCs at embryonic day 14.5 (E14.5) between arthritic and pregnancy-remitted mice. A selected target, serum amyloid A3 (SAA3), was further investigated using quantitative PCR (qPCR) and ELISA.

Results: Pregnancy resulted in complete or partial remission in 64% of the zymosan-treated SKG mice. 27 transcripts were differentially expressed in maternal PBMCs between arthritic and pregnancy-remitted mice (False Discovery Rate=0.05). Plasma SAA3 levels decreased with pregnancy-induced arthritis amelioration (one-way ANOVA; n=6, $p<0.05$) and correlated with arthritis severity (linear correlation; $R2=0.59$, $p<0.001$).

Conclusions: These results define a set of PBMC transcripts altered by pregnancy that correspond with remission providing insight into immune alterations. Further, these results establish SAA3 as a biomarker of arthritis amelioration in SKG mice. Future studies will further elucidate the molecular

and cellular mechanisms accounting for pregnancy-induced arthritis amelioration. *Supported by NIH/NCRR Colorado CTSI co-pilot award (LLD) and TL1 RR025778 (ALS); Arthritis Foundation Arthritis Investigator (LLD) and Postdoctoral Fellowship (LKP); UCD Dept. OB/GYN Academic Enrichment Fund (VDW).*

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The Effect of Vaginal Distention on the Smooth Muscle and Connective Tissue Proteins of the Mouse Urethra. Madeline A Dick-Biascoechea, Jie Xu, Kathleen A Connell, Nejla Sinclair, P Antonio Maldonado, Marsha K Guess. *Urogynecology and Reconstructive Pelvic Surgery, Yale University, School of Medicine, New Haven, CT, USA.*

Introduction: Stress urinary incontinence (SUI), one of the most common forms of incontinence, has been consistently linked to vaginal delivery. Vaginal distention (VD) is a proposed mechanism of injury during delivery leading to SUI. VD consists of stretching and displacement of vaginal muscles and surrounding tissues, including the urethra. VD has been associated with decreased leak point pressure (LPP) in the mouse urethra.

Objectives: To evaluate alterations in urethral tissue protein expression in the mouse urethra after VD compared to non-distended controls over time.

Methods: 66 female C57BL/6 mice were ovariectomized and supplemented with estradiol for 3 days. 35 mice had VD *via* transvaginal balloon catheter inflation while 31 mice received sham treatment. Mice were sacrificed at 0, 2, 3, 4, 7, 20 and 28 days after VD. Protein lysates were prepared and used for Western Blots (WB). Smooth muscle proteins alpha actin, calponin, smoothelin and SM22, and connective tissue proteins, collagens 1 and 3 and tropoelastin were analyzed with GAPDH as a loading control. WB were analyzed by densitometry with Image J and compared in Excel using Student's t-test. $P<0.05$ was statistically significant.

Results: All smooth muscle proteins showed a transient decrease in expression either immediately after VD or at day 2. Expression then increased as compared to the control group. These differences achieved significance in smoothelin on day 2 and calponin and SM22 on day 28. The connective tissue proteins collagen 1 and collagen 3 were both increased at all time points after VD compared to controls, achieving significance on day 0 and 28. Tropoelastin was more variable and no overall trend was observed.

Conclusions: Significant changes were observed in urethral smooth muscle and connective tissue proteins after VD. This suggests that urethral tissue remodeling occurs after VD to restore tissue properties and function to the pre-distended state. Aberrant or incomplete remodeling could contribute to decreased LPP and potentially, to the development of SUI.

Funding by NIH UL1RR024139 and RWJF Harold Amos Faculty Development Award.

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Bone Marrow-Derived Stem Cells Therapy for Injured Urethra of Female Rats. Michelle Z Ipolito, Rodrigo C Souza, Maria AT Bortolini, Marair MGF Sartori, Ismael DCG Silva, Manoel JBC Girao, Rodrigo A Castro. *Department of Gynecology, Federal University of São Paulo, Brazil.*

Introduction: Stress urinary incontinence (SUI) is the complaint of involuntary leakage on effort or exertion, or on sneezing or coughing. Pelvic floor trauma followed by vaginal delivery is the main risk factor for SUI development by causing urethral damage. Thus we hypothesize that (1) vaginal distension (VD) changes the histological composition and organization of the urethra; and (2) heterologous bone marrow-derived stem cells therapy may act to recover the injuries and restore the urethral structure of female rats after VD.

Methods: In these experiments, we analyzed the urethras of three groups of Wistar female rats: control, vaginal distention (VD) and VD receiving stem cell therapy. The balloon was inserted intravaginally in the groups of rats and inflated with 3ml of water for 12 hours intermittently. *In vitro* cultures were used for characterization and proliferation of the bone marrow-derived stem cells (MSC) from healthy female rats expressing green fluorescent protein (GFP) before the treatment. GFP cells were checked for authenticity and presented positive staining for MSC markers CD73, CD90, CD105, while negative staining for the CD34, CD45 and CD31. MSCs differentiated into adipocytes, chondroblasts and osteoblasts. After 72 hours of injury, we injected stem cells into the tail vein of the traumatized rats. GFP cells localization as well as urethral tissue morphologies were checked at days 7, 14, 21 and 28 after injection using IHC and electron microscopy.

Result: The rats had the VD layer of smooth and striated muscle with less thickness than the control rats confirmed by histological analysis. The VD rats treated with stem cells had more capillaries in the layer of striated muscle than VD rats and the control group. These capillaries mostly contained GFP tagging. We have noted the early presence of MSC in all layers of the urethra of rats after 7 days of therapy. The cells showed GFP phenotyping of smooth and striated muscle during 28 days.

Conclusion: We have developed an animal model of urethral injury throughout VD that persists to 28 days. Alterations in the components and structure of the damaged urethra may suggest that stem cells regenerate tissue. These findings may encourage additional studies addressing the potential role of stem cell therapy in SUI.

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Impact of Parity on Smooth Muscle Contractility. Zegbeh C Jallah,[2] Naoki Yoshimura,[3] Steve D Abramowitch,[2] Pamela A Moalli.[1] *[1]Division of Urogynecology & Reconstructive Pelvic Surgery, Magee Womens Research Institute, Pittsburgh, PA, USA; [2]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Urology & Pharmacology, Univerisity of Pittsburgh School of Medicine, Pittsburgh, PA, USA.*

Introduction: Parity is a known risk factor for the development of pelvic organ prolapse (POP); but the mechanism remains unknown. In women with prolapse, the amount of vaginal smooth muscle (VASM) is decreased, yet POP is often treated with synthetic polypropylene meshes which have been shown to cause significant disruption of VASM contraction, thus impacting sexual function, vaginal tone, and compliance. Our objective was to determine the impact of parity on VASM function, as a risk for the development of POP.

Methods: Within 30 mins of excision, the vagina from virgin (N=6) and parous (N=6) sheep were placed into oxygenated Krebs'-buffer. Circumferential strips (5x7mm) from the proximal, mid- and distal vagina were suspended in tissue baths monitored with a pressure transducer. Each strip was preloaded to 1g and equilibrated (60 mins), while recordings were obtained at a 40Hz rate. Contractile force in response to non-cumulative dosages of carbachol (10^-7 - 10^-4 M) and KCl were normalized to strip volume (mN/mm3), and statistically compared using Students T-test ($P<.05$).

Results: The dose response curves and the maximum force generated in response to KCl, were similar in all vaginal regions, although the response of the mid-vagina was higher (insignificantly) in both the virgin and parous sheep (P=0.06, 0.11). There was a two-fold increase in contractile force in the proximal, mid-, and distal vagina of the parous sheep, relative to the virgin sheep (P=0.001, 0.01, 0.03). The increase was also evident in the contractile response to carbachol, in the distal and mid-vagina (P=0.01, 0.001), but not the cholinergic dominant proximal vagina (P=0.13).

Conclusions: Overall muscle and receptor mediated contraction of circumferential SM increased in all regions of the vagina with parity, consistent with data of SM injury following vaginal delivery. These and previous morphometric data showing decreased smooth muscle volume in women with prolapse, support a temporal sequence in which initial injury to VASM progresses with the development of prolapse and is further compromised following treatment with a synthetic mesh. Future materials that restore instead of compromise SM function should be developed.

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Procollagen N Proteinase Expression in Vaginal Tissue of Women with and without Pelvic Organ Prolapse. Hala Kufaishi,[1] Oksana Shynlova,[1] Harold Drutz,[2,3] Stephen Lye,[1,2] May Alarab.[1,2,3] *[1]Samuel Lunenfeld Res Inst, Mt Sinai Hospital, Toronto; [2]Dep of Ob/Gyn, Univ of Toronto; [3]Div of Urogynecology, Mount Sinai Hospital, Toronto, Canada.*

Hypothesis /Aims of Study: Pelvic Organ Prolapse (POP) is a result of failure in the pelvic floor support and abnormal connective tissue may play a role in its etiology. ADAMTS-2,-3 and -14 are extracellular procollagen-N-proteinases, that cleave the N-propeptides of procollagens I, II, III in vivo. This effectively converts fibrillar procollagens to mature collagen monomers. Mutations in ADAMTS-2 were identified in patients with Ehlers Danlos Syndrome. We hypothesized that ADAMTS-2, -3 and -14 genes and proteins are differentially expressed in women with severe POP compared to healthy premenopausal women.

Material and Methods: Premenopausal Caucasian women undergoing total hysterectomy for benign conditions were recruited as controls (POP-Q = 0) while women with advanced POP (POP-Q stage ≥ 3) undergoing vaginal hysterectomy were recruited as patients. Patients and controls were further divided based on phase of menstrual cycle (proliferative vs. secretory) confirmed by histology report. Exclusion criteria: steroids therapy, malignancy, previous pelvic surgery, connective tissue diseases. During surgery 1 cm^2 of full

thickness vaginal tissue was obtained; total RNA and protein were extracted and analyzed by RT-PCR and Western Immunoblot, respectively. Mann-Whitney test ($p<0.05$) was used for statistical analysis.

Results: We recruited 40 Caucasian premenopausal women (20 patients and 20 controls). ADAMTS-2 mRNA was expressed in all vaginal biopsies and was significantly increased in all patients with POP compared with all controls. ADAMTS-3 and ADAMTS-14 was found in vaginal tissue of controls, but not in POP patients. Four bands corresponding to pro and mature forms of the two major isoforms of ADAMTS2 (active and inactive) were detected on Immunoblot: 130 kDa, 100 kDa, 58 kDa and 34.5 kDa. The 58 kDa protein was significantly decreased in patients vs controls only in the proliferative phase of the menstrual cycle ($p=0.027$). Furthermore, we noticed that both (pro and mature) forms of inactive ADAMTS-2 protein were noticeably more abundant than their active counterparts. **Conclusion:** Young patients with severe POP showed altered expression of ADAMS-2 gene and protein. These changes may contribute to a deficient vaginal connective tissue and support.

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LOX, TIMP and MMP Enzymes Expression in Vaginal Tissue of Premenopausal Women with and without Pelvic Organ Prolapse. Hala Kufaishi,[1] Oksana Shynlova,[1] Harold Drutz,[2,3] Stephen Lye,[1,2] May Alarab.[1,2,3] *[1]Samuel Lunenfeld Res Inst, Mt Sinai Hospital, Toronto; [2]Dep of Ob/Gyn, Univ of Toronto; [3]Div of Urogynecology, Mt Sinai Hospital, Toronto, Canada.*

Hypothesis /Aims: Evidence suggests that abnormalities of connective tissue structure or its repair mechanism may predispose women to Pelvic Organ Prolapse (POP). We reported earlier that the Lysyl oxidase (LOX) family gene and protein expression involved in the maturation of collagen and elastin was decreased in the vaginal tissue of premenopausal women with severe POP in the proliferative phase of the menstrual cycle. Here we examined the expression of proteins involved in elastin and collagen metabolism, namely LOX, LOXL1-4, TIMP 1-4, and MMP1,2,7,9,12,14 in the vaginal tissue of premenopausal women with advanced POP and asymptomatic controls in the secretory phase of the menstrual cycle.

Methods: Premenopausal Caucasian women undergoing total hysterectomy for benign conditions were recruited as controls (POP-Q = 0) while women with advanced POP (POP-Q stage ≥ 3) undergoing vaginal hysterectomy were recruited as patients. Secretory phase of the menstrual cycle was confirmed by histology report. Exclusion criteria: steroids therapy, malignancy, previous pelvic surgery, connective tissue diseases. During surgery, 1 cm^2 of full thickness vaginal tissue was obtained, total RNA and protein were extracted and analyzed by RT-PCR and Western Immunoblot, respectively. Mann-Whitney test ($p<0.05$) was used for statistical analysis.

Results: 18 women were enrolled (8 patients and 10 controls). LOX, LOXL1-4 gene were expressed in vaginal tissue biopsies, however only LOX was significantly increased ($p=0.0028$). We detected a significant increase in MMP2 and MMP14 mRNA levels in patients with POP compared to controls ($p=0.029$, $p=0.008$ respectively). Immunoblot analysis for LOX and MMP14 indicated a significant increase of the 35kDa form of LOX in vaginal tissue of patients compared to controls ($p=0.04$) however two isoforms of MMP14 were expressed equally. MMP-2 and -9 gelatinase activities were significant increased in patients compared to controls.**Conclusion:** We identified differential expression of proteins that may contribute to altered collagen and elastin biogenesis and subsequent defective assembly of pelvic tissues in patients with severe POP in the secretary phase of the menstrual cycle. Hormonal status might influence the expression of enzymes regulating the ECM biogenesis in vaginal tissue.

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HomeoboxA11 (HOXA11) Knockdown Alters Cell Proliferation and Apoptotic Signals in Primary Human Uterosacral Ligament Cell Cultures. Yan Ma, Akshitar Datar, Yingqun Huang, Kathleen A Connell. *Obstetrics, Gynecology & Reproductive Sciences, Yale University, School of Medicine.*

Pelvic organ prolapse (POP) is a common, debilitating disorder associated with attenuated uterosacral ligaments (USLs). We have reported that HOXA11, a conserved homeobox gene, is essential for development of USLs and is deficient in USLs of women with POP. We have also found decreased cellularity in prolapsed USLs and that HOXA11 overexpression in vitro increases fibroblast proliferation. Others reported increased apoptosis in prolapsed USLs. Further, Hoxa11 knockdown (KD) in murine USLs decreases collagen expression. We hypothesized that HOXA11 KD in primary human USL (PHUSL) cell culture alters cell proliferation and/or apoptosis. PHUSL cells were generated from USLs of two women without POP and transfected with either control plasmid or HOXA11 siRNA. RNA was extracted at 24hr and 48hr and protein at 48hr and 72hr post-transfection. mRNA levels of HOXA11, p53, tumor necrosis factor (TNF)α, transforming growth factor (TGF)β1, acidic and basic fibroblast growth factor (aFGF and bFGF) were measured via realtime-PCR. Protein expression of HOXA11, phosphorylated (p)-p53, TGFβ1, p-p38 mitogen-activated kinase (p-p38MAPK), bFGF, and p-p44/p42 MAPK (or p-Erk1/2) were measured via westernblot. HOXA11 mRNA levels decreased 79% and protein expression decreased 20% at 48hr. mRNA expression of TNFα ($P<0.05$) was upregulated and p53 ($P<0.05$), aFGF ($P<0.05$) and bFGF ($P=0.07$) downregulated at 48hr. Although the increase in TGFβ1 mRNA level at 48 hr was not significant ($P=0.09$), its protein expression increased >4-fold at both 48hr and 72hr ($P<0.05$). Despite decreased p53 mRNA expression, p-p53 increased at 48hr ($P=0.09$), reaching significance at 72hr ($P<0.05$). p-p38MAPK increased at both 48hr and 72hr ($P<0.05$). In contrast, bFGF protein expression decreased by 44% at 48hr ($P<0.05$) and p-Erk1/2 decreased by 37% at 72hr ($P<0.05$). In summary, HOXA11 KD promoted cell apoptotic signals suggested by increased TNFα, TGFβ1, p-p53 and p-p38MAPK expression, and repressed proliferative signals suggested by decreased p-Erk1/2, aFGF and bFGF expression in PHUSL cells. These data, along with previous findings of decreased cellularity and increased apoptosis in prolapsed USLs, and decreased collagen expression after Hoxa11 KD in murine USLs, suggest that HOXA11-mediated pathways are involved in maintaining cell population and collagen synthesis, which is important to the USL tissue strength.