1

Pre-Treatment with a Toll-Like Receptor 4 Antagonist Inhibits Lipopolysaccharide-Induced Preterm Uterine Activity, Cytokines, and Prostaglandins in a Non-Human Primate Model. Kristina Adams,[*1,2,3] David Persing,[4] Drew Sadowsky,[*3] Miles Novy,[*3] David Soergel,[5] Michael Gravett.[*1,3]
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Objective: The majority of early preterm births are complicated by intrauterine infection and associated with activation of innate immune responses resulting in production of cytokines and prostaglandins. We hypothesized that blockade of the innate immune response to lipopolysaccharide (LPS) by intra-amniotic infusion of a Toll-like receptor 4 antagonist (TLR4A) would prevent LPS-induced preterm uterine activity and elevations in amniotic fluid (AF) cytokines and prostaglandins.

Methods: Fourteen chronically catheterized pregnant rhesus monkeys (*Macaca mulatta*) at 128-147 days gestation (term=167 days) received one of three intra-amniotic infusions: 1) saline (n=6), 2) 1-10 µg LPS (n=4), or 3) TLR4A pre-treatment followed by 10 µg LPS (+1 hour; n=4). Uterine activity (hourly contraction area) and AF leukocytes (WBC), cytokines (IL-1β, IL-6, IL-8, TNF-α), and prostaglandins (PGE_2, $PGF_{2\alpha}$) were serially measured before and after inoculations. Stastistical analysis compared peak post infusion uterine activity or AF cytokine, PG or WBC levels between groups and was performed on log transformed data using oneway ANOVA followed by pairwise comparisons adjusted using the Bonferroni method.

Results: Intra-amniotic infusion of LPS alone induced significant elevations in uterine activity and AF WBC, IL-8, TNF-α, PGE_2, and $PGF_{2\alpha}$ compared to saline controls (all $p<0.05$). In contrast, pre-treatment with a TLR4A was associated with no significant increases in uterine activity, cytokines, or prostaglandins when compared to saline controls. Treatment with a TLR4A was also associated with significantly lower AF WBC, IL-8, TNF-α, PGE_2, and $PGF_{2\alpha}$ compared to LPS infusion alone (all $p<0.05$).

Conclusions: Pre-treatment with a TLR4A inhibited LPS-induced uterine activity, leukocytes, cytokines and prostaglandins in non-human primates. These data indicate that treatment with toll-like receptor antagonists, together with antibiotics, may delay or prevent infection-associated preterm birth.

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2

Rhesus Monkey Early Placental Vascularization, Growth and Development after Anti-Mamu-AG Passive Immunization. Gennadiy I Bondarenko,[1] David W Burleigh,[1] Maureen Durning,[1] Thaddeus G Golos.[1,2] (SPON: Ronald R Magness). *[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA; [2]Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI, USA.*

Objective: The rhesus monkey placenta expresses the nonclassical MHC class I molecule, Mamu-AG, which has HLA-G-like characteristics. These molecules primarily are expressing in the placenta and thought to play a role in maternal-fetal immune interaction. The aim of this study was to determine how anti-Mamu-AG passive immunization affects placental growth and development in the nonhuman primate.

Methods: We conducted histological, morphometric and immunohistochemical (cytokeratin, SMA, Von Willebrand factor, VEGF, Flk-1, Ki-67 expression) analysis of placentas on day 24 of gestation of 12 rhesus monkeys (Macaca mulatta) with normal pregnancy (Untreated control), treated by 4 mg non-specific purified F(ab')2 fragments (Non-specific treated) or with 4 mg anti-Mamu-AG (clone 25D3) mAb (25D3-treated) intravenous per day from day 18 through day 24 of gestation.

Results: On day 24 in 25D3-treated placentas vs Untreated and Non-specific treated groups we found a delay in gestational changes of histological features of implantation (degeneration of epithelial plaque, decline of edema, decidualization, lacunae organization). On the same day small arteries directly beneath the implantation site were completely occluded by extravillous cytotrophoblasts in all animals. The arterial endothelial layer in all groups and the tunica media in Untreated and Non-specific treated groups breached, but in 25D3-treated placentas the tunica media remained intact. The number of villous cross sections per mm2 of paraffin section, length of stem villi and diameter of all villi were significantly less in 25D3-treated placentas. The proliferative index (Ki-67-positive cells) dramatically decreased in anti-Mamu-AG passive immunized monkeys compared to Untreated and Non-specific-treated groups. The number of vessels per villi, diameter of villous vessels and Flk-1 expression in chorionic villi were less in 25D3-treated placentas.

Conclusions: We propose that the anti-Mamu-AG-passive immunization in early gestation down-regulates trophoblastic modification of decidual vessels, placental vascularization, growth and development. This data suggest an important interaction between placental MHC class I expression and an appropriate enviroment for placental and vascular development in primate pregnancy.

3

Expression of Spermatogenesis-Related Retrogenes in Ovarian Cancer. Matthew L Anderson,[*1] Russell Broaddus,[2] Colin Bishop,[1] Jan Rohozinski.[1]
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BACKGROUND: During spermatogenesis, activation of a family of retrogenes with X-linked progenitors compensates for transcriptional inactivation of the X chromosome at meiosis. We recently found that one such retrogene, *UTP14C*, is expressed in ovarian cancers at high frequency. We have extended this work to determine if other spermatogenesis-related retrogenes are also transcriptionally active in ovarian cancers. **METHODS:** Total RNA was extracted from ovarian cancers using TRIzol (Invitrogen, Inc). After treating each specimen with DNAase, cDNA was synthesized with reverse transcriptase. Polymerase chain reaction (PCR) was used to assess the expression of specific gene products from *BIRC8, RPL10L* and *TAFIL*. Expression of the corresponding X-linked progenitors (*BIRC4, RPL10, TAFI*) was also tested. **RESULTS:** We observed robust expression of each X-linked progenitor (*BIRC4, RPL10, TAF1*) in all somatic tissues, normal human testis, pre and post-menopausal ovaries and most ovarian cancers tested. In contrast, expression of the complementary retrogenes was observed in testis and, in one case, premenopausal ovary, but was notably absent in the 17 different somatic tissues tested. These retrogenes were also frequently expressed in ovarian cancers of different histologies. **CONCLUSIONS:** We conclude that the expression of functionally distinct members of the spermatogenesis-related retrogene family occurs at high frequency in epithelial ovarian cancers. Given the near-uniform absence of their expression in somatic tissues, these retrogenes offer potentially ideal diagnostic and/or therapeutic targets for ovarian cancer. Their expression may also indicate specific sites of X chromosome inactivation that may be important in the pathogenesis of this disease.

Frequency of Retrogene Expression in Ovarian Cancers

Tumor Histology	BIRC8	RPL10L	TAF1L
Papillary Serous	43/55 (78%)	41/58 (71%)	32/58 (55%)
Mixed	11/18 (61%)	13/18 (72%)	8/17 (47%)
Endometrioid	3/4 (75%)	3/4 (75%)	2/9 (22%)
Clear Cell	2/3 (33%)	3/4 (75%)	3/9 (33%)
AGCT	2/3 (67%)	3/3 (100%)	2/5 (40%)

4

Metformin Reverses Adverse Effects of IGF-1 on Blastocysts. Emily Jungheim, Grace Eng, Rachael Sheridan, Kristin Bibee, Kelle Moley.[*]
Obstetrics and Gynecology, Washington University, St. Louis, MO, USA.

Hypothesis:IGF-1 exposed blastocysts show decreased insulin-stimulated glucose uptake and increased apoptosis. Metformin activates AMPK, reversing IGF-1's effects and improving pregnancy outcome.

Methods:2-cell embryos collected from superovulated mice were cultured for 72° to blasocysts in 1 of 4 treatments: control human tubal fluid (HTF), 25ug/mL metformin, 200nM IGF-1, or metformin+IGF-1. Enzymatic assays for non-radioactive deoxyglucose uptake were performed on insulin(500nM)-stimulated blastocysts. Apoptosis was detected by confocal microscopy using a TUNEL assay and Topro-3 nuclear dye. Embryos were scored for %TUNEL positive/total nuclei. Fetal resorption rates and crown rump lengths(CRL) were determined at embryonic day 14.5 after transfer of blastocysts from each treatment group into uterine horns of recipient mice. AMPK activation by metformin was analyzed by western blot with anti-phospho-AMPK using 2-cell embryos cultured for 72° in HTF or metformin+HTF.

Results: Metformin exposed blastocysts showed a 2.3X increase in AMPK phosphorylation(n=3 experiments). Insulin-stimulated glucose uptake was lower in blastocysts cultured in IGF-1(-0.16±0.11mmol/kg wet weight/15minutes;n=55) vs HTF(0.29±0.13;n=47;$p<0.05$) and vs metformin+IGF-1(0.25±0.11;n=55;$p<0.01$). No difference was seen among metformin+IGF-1 vs HTF or metformin only groups(0.068±0.03;n=45). Metformin reversed apoptosis in IGF-1 exposed blastocysts(11.0±1.6 IGF-1 vs. 5.8±0.9 metformin+IGF-1; n=28; $p<0.001$). Apoptosis in IGF-1+metformin treated embryos was not different than HTF(n=21;7.6±1.2) or metformin only(n=21;4.0±0.8)). Exposure to excess IGF-1 showed lower implantation rates at embryonic day 14.5(34.7±2;n=8 experiments;$p<0.003$) vs culture in

HTF(73.3±3.9;n=8). Implantation rates were higher with IGF-1+metformin co-culture(63.0±9.8;n=8;p<0.05). Addition of metformin decreased resorption rates from 46.3±11 in the IGF-1 group to 24.7±4.3. CRL was shorter for the fetuses in the IGF-1 group(n=53) than the HTF group(n=71)(81.5±16mm vs 110.0±15;p<0.05), and the metformin+IGF-1 group(100.1±11;p<0.05).

Conclusions: Women with PCOS are at higher risk for miscarrying. The mechanisms responsible are not clear, but increased IGF-1 levels may play a part by leading to decreased AMPK activity. The AMPK activator metformin is widely used to improve ovulation in women with PCOS. These data provide evidence for metformin use beyond ovulation induction to improve pregnancy outcome.

5

Pregnancy Outcome in Relation to Oral Diseases and Attitudes. A Multicentric Study. Silvio Abati,[1] Paola Pileri,[2] Guglielmo Campus,[3] Livia Ottolenghi,[4] Stefania Calabrese,[2] Anna Martinelli,[2] Salvatore Dessole,[4] Laura Strohmenger,[1] Irene C Cetin.[*2] *[1]Dental Clinic, Dept. of Medicine, Surgey, Dentistry, University of Milano, Milano, Italy; [2]Inst. of Obstetrics and Gynecology, University of Milano, Milano, Italy; [3]Inst. of Dentistry, University of Sassari, Sassari, Italy; [4]Dept. of Dental Sciences, University of Rome La Sapienza, Rome, Italy; [5]Dept. of Pharmacology and Gynecology, University of Sassari, Sassari, Italy.*

Objectives. Periodontal disease could play a role during pregnancy as a source of chronic infection having so the potential to be the direct cause of an adverse effect, both on the mother, the placenta and the fetus. The aim of this study was to determine whether oral health conditions and attitudes correlate with adverse fetal and/or maternal outcomes. Methods. A multi-centric cross-sectional study was designed. 304 women were recruited and examined within 5 days postpartum through a standardised questionnaire and a full oral and periodontal examination. Case subjects (n=62) had premature delivery (n=25) and/or delivered SGA/IUGR (n=25) and/or experienced Preeclampsia /PIH (n=18) or PROM (n=6) during their pregnancies whereas control subjects (n=242) had normal pregnancies, delivered at or around term and had newborns with normal weights. Results. Cases and controls were similar in mean age, birthplace, ethnicity, educational level, smoking habits and in oral hygiene habits. The analyses of the periodontal health and disease variables (bleeding on probing, tooth attachment loss and probing depth score) showed no relevant differences between the two groups. There was no differences in proportion of smoking women in cases and controls (chi square = 0.3, p=0.60). No significant association was observed regarding cases and the level of periodontal disease (chi square for trend =1.19, p=0.27). The mean proportion of periodontal sites probing more than 4 mm observed in the case subjects compared to the controls failed to reach statistical significance. Conclusions. Our preliminary report failed to demonstrate a positive association between periodontal disease and adverse pregnancy outcomes. However, our study population is different from previously reported case-control populations in both demographic factors and the level of periodontal disease. More data need to be collected in order to exclude the proposed potential link between periodontitis and the risk of pregnancy complications. (Supported by the MUR-PRIN 2005 grant).

6

Thrombin-Induced Changes in Contraction Associated Protein Expression in Pregnant Term Myometrium. David N Hackney,[1,2] Jye-Ping Chiao,[1,2] Suzanne E Peterson,[1,2] Hyagriv N Simhan.[*1,2] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh/Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetric-Fetal Pharmacology Research Units Network, Bethesda, MD, USA.*

Objective: Thrombin has been demonstrated to stimulate contractions and increase intracellular calcium in myometrium *in vitro*. Its ability to increase the expression of contraction associated proteins (CAPs) in myometrium remains unexplored. The hypothesis of this study is that myometrial cells incubated with thrombin will increase their expression of oxytocin receptor (OTR), COX-II and Connexin-43.

Methods: Samples of myometrial tissue were collected from pregnant patients at term undergoing cesarean sections. After tissue cultures were established, the cells were incubated with varying concentrations of thrombin for 16 hours. The relative protein concentrations of OTR, Cox-II and Connexin-43 were then determined through Western blot analysis. The results were normalized to control. The results from samples which had been exposed or not exposed to thrombin were analyzed with Mann-Whitney two sample rank sum tests when the results were non-parametrically distributed and the t-test when the distributions were normal.

Results: Table 1 contains the relative concentrations of the CAPs in response to different thrombin concentrations, normalized to control. A statistically significant increase in expression was found between the control samples and the cells exposed to thrombin for OTR (p=0.009) and Connexin-43 (p=0.001). COX-II demonstrated an inverse U concentration relationship.

Conclusion: Thrombin increases the expression of CAPs in cultured myometrial cells. This response may play a role in the development of labor following intrauterine bleeding.

CAP expression normalized to control

Thrombin U/mL	0.5	1.0	2.5	5.0	10	25
OTR	2.71	2.42	2.55	4.59	4.74	3.97
COX-II	1.44	1.25	0.93	0.55	0.56	0.34
Connexin-43	2.18	2.71	2.36	2.47	1.54	1.94

7

Expression of Protease Activated Receptor (PAR) Subtypes in Human Myometrium and Decidual Cells. David N Hackney,[1,2] Jye-Ping Chiao,[1,2] Suzanne E Peterson,[1,2] Hyagriv N Simhan.[*1,2] *[1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh/Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetric-Fetal Pharmacology Research Units Network, Bethesda, MD.*

Objective: Thrombin and trypsin have been demonstrated *in vitro* to stimulate contractions and increase intracellular calcium in myometrium and the expression of cytokines, prostaglandins and other products from decidual tissue. Four subtypes of the protease activated receptor exist (PAR-1,2,3,4), the relative expression of which have not yet been fully characterized in human myometrial and decidual cells. Studies of myometrium derived from rats suggest that PAR-2, 3 and 4 are minimally expressed, especially in non-pregnant samples. The objective of this study was to characterize the expression of the PAR subtypes in human myometrium and decidua.

Methods: Cell cultures were derived from myometrial samples collected from term pregnant patients undergoing cesarean sections, non-pregnant patients undergoing hysterectomy and decidual cells from term placentas. Immunohistochemistry was performed utilizing commercially available (Santa Cruz Biotechnology, Inc) primary antibodies with specificity to the four PAR subtypes.

Results: Term pregnant and non-pregnant myometrium both expressed all four PAR subtypes in a cytoplasmic distribution. The staining intensity for PAR-2 and 4 appeared to be similar among the pregnant and non-pregnant cells. Decidual cells expressed PAR-1,2 and 4. Staining for PAR-3 was equivocal in the decidual cells.

Conclusions: All four subtypes of the PAR receptor are expressed in cell cultures derived from both pregnant and non-pregnant myometrium. This is in contrast to data from myometrium derived from the rat. All subtypes are also expressed in decidual cells in culture, with the possible exception of PAR-3.

8

Leukocyte Activation in Peripheral Blood during Pregnancy. Meifang Yuan,[1] Iain B McInnes,[2] Margaret M Harnett,[2] Jane E Norman.[*1] *[1]Reproductive and Maternal Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Inflammation, Infection and Immunity, University of Glasgow, Glasgow, United Kingdom.*

Background: Inflammatory activity is suppressed during normal pregnancy and a Th1 to Th2 shift can be demonstrated. In contrast, parturition has been likened to an inflammatory reaction with evidence that leukocytes play a crucial role.

Objective: Inappropriate preterm activation of leukocytes may lead to preterm delivery. We hypothesised that leukocytes are primed and activated in peripheral blood during parturition.

Methods: Whole peripheral blood was analysed by flow cytometry to assess surface markers of leukocyte activation using activation-associated antibodies. Samples were taken from 30 pregnant women: 28-34 week gestation preterm not in labour (PTNL) n=10, 37-42 week gestation term not in labour (TNL) n=10 and 37-42 week gestation term in labour (TL) n=10. The absolute cell numbers of leukocyte subpopulations were determined using a Sysmex KT-21 analyzer from 20 women in each group. Student's t-test was used for statistical analysis.

Results: There were more granulocytes in the TL group than in the PTNL or TNL groups (P<0.001, Table I) but no differences could be detected in the lymphocyte and monocyte populations. The expression levels of CD11b on the surface of monocytes from TL were significantly higher (P<0.05) than those of PTNL or TNL. CD26 and CD28 expression levels on lymphocytes from TL were significantly higher (P<0.05) when compared with PTNL (Table II).

Conclusions: Parturition appears to modify leukocyte subpopulations. Circulating leukocytes from labouring women showed greater expression of adhesion molecules and activation markers. These findings contribute to the concept that parturition is an inflammatory event. However, the precise mechanism by which leukocyte activation is regulated, and its' function is still unclear.

Distribution of Leukocyte Subpopulations

	Lymphocytes	Monocytes	Granulocytes
PTNL	2.18±0.71	0.30±0.18	7.34±2.27
TNL	2.21±0.72	0.33±0.14	7.29±1.88
TL	1.98±0.77	0.39±0.10	11.2±1.91***

The absolute cell number expressed as million cells/ml. ***P<0.001, as compared with PTNL or TNL.

Surface Activation Marker Expression

	CD11b (for Monocytes)	CD26 (for lymphocytes)	CD28 (for lymphocytes)
PTNL	88.03±5.19	57.60±14.7	87.67±5.4
TNL	87.34±5.96	70.87±7.13*	90.83±6.19
TL	92.92±4.49**	71±13.7*	93.37±3.93*

**P<0.05, as compared with PTNL and TNL, *P<0.05, compared with PTNL.

9

Characterisation of Class I and Class II HDACs in the Human Myometrium in Pregnancy. Kelly A Harper, Stephen C Robson,* Gary N Europe-Finner, Alison J Tyson-Capper. *Surgical & Reproductive Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Objectives: Histone deacetylases (HDACs) are known to play a part in the remodelling of chromatin at specific promoters. We aimed to a) characterise the expression of class I and class II HDACs and investigate their activity in the non-pregnant, term pregnant and labouring myometrium using samples from the upper and lower regions of the uterus, and b) determine the effect of the HDAC inhibitor trichostatin A (TSA) on the expression of COX-2 and other inflammatory proteins in myometrial and amnion cells.

Methods: Western immunoblotting and immunofluorescence were employed to characterise HDAC expression. HDAC activity was determined using a colorimetric activity assay system. Cultured myometrial and amnion cells were treated with TSA in the presence of LPS to determine the effect of TSA on COX-2 activation, levels of which were analysed at 0, 6, 12 and 24 hours following treatment. Similarly, levels of the transcription factor c-jun and DNA methyltransferase 1 (Dnmt-1) were also determined.

Results: Distinct temporal and spatial patterns of expression were observed for the individual HDACS, 1,2,3,4,6 and 8 within these tissues. A substantial reduction in expression of all HDACs was observed in term non-labouring samples when compared to non-pregnant samples whereas there appeared to be an increase in expression of class l HDACs, in particular HDACs 1, 2 and 8 in labouring biopsies taken from the upper uterine region. Our data also indicates that TSA inhibits COX-2 activation in myometrial and amnion cells at the protein and mRNA level and down-regulates expression of c-jun and Dnmt-1.

Conclusion: The differential expression of known HDACs in the upper and lower uterine segments may be important in the epigenetic regulation of myometrial genes associated with contractility during parturition. In addition to its known role in altering acetylation we also show that TSA may affect DNA methylation. Further studies are now underway to determine the effect of TSA and other HDAC inhibitors on HDAC expression/acetylation status and other pro-inflammatory genes that are associated with normal and preterm labour.

10

Placental Expression of Alpha Dystroglycan. Manjula Santhanakrishnan, Karen Oppenheimer, Elizabeth A Bonney.* *OB/GYN, University of Vermont College of Medicine, Burlington, VT, USA.*

Background: Several aspects of viral infection of the placenta including mechanism and consequences of the fetus are unclear. While local immunity is likely to be an important regulator of this phenomenon, it is also likely that both inherent placental and viral factors play an important role. Infection of pregnant mice with Lymphocytic choriomeningitis virus (LCMV) results in placental infection, and thus we sought to determine possible placental receptors for this virus. Alpha- dystroglycan (α-DG), a component of the Dystroglycan complex, is expressed in many organs at differing levels. It plays a role in a number of essential physiologic processes, and has been identified as a receptor for LCMV.

Objective: To observe expression levels of placental α-DG.

Methods: Three month old C57BL/6 female mice under went timed mating with same-strain males. On days 10,14,16,17 and 18 of gestation pregnant females were euthanized. A representative placenta from each mother was carefully separated from the uterus and fetus and lysed in RLT buffer. Total RNA was isolated (Qiagen's RNAEasy kit) and cDNA was made. Real time PCR was performed with exon-spanning primers specific for α-DG using SYBR green chemistry on an ABI prism 7000. mRNA expression levels were determined using the relative standard curve method and by normalizing to expression of β-2 microglobulin. Western blot analysis was performed on lysed whole placental protein using a specific anti α-DG antibody (Upstate).

Results: Using quantitative RT-PCR we observed expression of placental α -DG from day 10 – 18 of pregnancy. As compared to day 10 placentas (n=3), we observed a two-fold decrease in expression in placentas from day 14 (n=4 p<0.02). Expression was observed to return back to day 10 levels in placenta from day 18 (n=4, p=0.428). In a separate experiment, we found that day 16 placenta isolated from different locations in the uterus (near the ovary, mid-horn and near the cervix) of the same mouse showed similar levels of α-DG expression. The western blot confirmed expression of α-DG throughout gestation.

Conclusion: α-DG expression is constitutively present in placenta throughout pregnancy, and this is consistent with the idea that α-DG could be a placental receptor for LCMV. Expression levels are high during day10 and day18 and this may predict the days of gestation during which the susceptibility to infection by the virus is also increased.

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11

"The Inflammasome" in Human Parturition. Beth L Pineles,[1] Roberto Romero,*[1,2] Daniel Montenegro,[1] Adi L Tarca,[1,3] Nandor Gabor Than,[1] Sonia Hassan,[1,4] Francesca Gotsch,[1] Sorin Draghici,[3] Jimmy Espinoza,[1,4] Chong Jai Kim.[1,5] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [3]Department of Computer Science, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [5]Department of Pathology, Wayne State University, Detroit, MI, USA.*

OBJECTIVE: "The inflammasome" is the first line of immune response to cell stress (microbial products or endogenous danger signals: Cell 2006, 126:659). This intracellular protein complex leads to activation of caspase 1 and evokes the cleavage of pro-IL-1β, pro-IL-18, and pro-IL-33 to their mature secreted forms. Three proteins form part of the inflammasome: NALP1, NALP3 and Ipaf. Genome-wide studies have identified that an acute inflammatory signature characterizes spontaneous parturition (AJOG 2006, 195:394). The aim of this study was to determine whether the expression of the major inflammasome components changes in maternal peripheral blood during spontaneous labor at term.

METHODS: Maternal peripheral blood was collected from patients with normal pregnancies who delivered at term with (n=9) and without labor (n=8). The mRNA expression of Ipaf, NALP1, and NALP3 was determined by real-time qRT-PCR. Generalized estimating equations were used to derive p-values.

RESULTS: Patients with spontaneous labor at term had higher mRNA expression of Ipaf than patients who were not in labor (p<0.01). In contrast, there were no differences in NALP1 or NALP3 mRNA expression between the two groups.

CONCLUSIONS: The differential expression of Ipaf mRNA provides novel *in vivo* evidence for the involvement of the inflammasome in parturition. This protein complex may play a critical role in parturition as well as in host response by inducing maturation of IL-1β, IL-18 and IL-33.

12

Differential Expression of the Inflammasome Components in the Fetal Inflammatory Response Syndrome. Daniel Montenegro,[1] Roberto Romero,*[1,2] Beth L Pineles,[1] Adi L Tarca,[1,3] Sally A Madsen-Bouterse,[1] Sonia Hassan,[1,4] Juan Pedro Kusanovic,[1] Sorin Draghici,[3] Jimmy Espinoza,[1,4] Chong Jai Kim.[1,5] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [3]Department of Computer Science, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [5]Department of Pathology, Wayne State University, Detroit, MI, USA.*

OBJECTIVE: "Inflammasomes" are caspase 1-activating complexes which include the NOD-like receptors Ipaf, NALP1, and NALP3. These proteins detect microbial products and endogenous danger signals, resulting in the release of IL-1β and IL-18 by monocytes and macrophages. Maternal and

fetal inflammation (chorioamnionitis and funisitis, respectively) are frequent complications of pregnancy. A systemic fetal inflammatory response syndrome (FIRS) is associated with increased perinatal morbidity and long-term handicap. The aim of this study was to determine whether chorioamnionitis and FIRS are associated with changes in the mRNA expression of Ipaf, NALP1, and NALP3 in maternal peripheral and umbilical cord blood.

METHODS: Leukocyte RNA was isolated from maternal and cord blood collected into PAXgene blood RNA tubes from patients with no evidence of inflammation (no chorioamnionitis, no funisitis and cord plasma IL-6 <11 pg/ml; n=10) and those with chorioamnionitis and FIRS (funisitis and cord plasma IL-6 >11 pg/ml; n=10). All patients had spontaneous preterm labor. Ipaf, NALP1, and NALP3 mRNA expression in peripheral blood was determined by real-time qRT-PCR. Generalized estimating equations were used to derive p-values.

RESULTS: 1) mRNA expression of Ipaf in maternal and cord blood was higher in patients with chorioamnionitis and FIRS than in those without inflammation ($p<0.01$); 2) In contrast, mRNA expression of NALP1 was lower in patients with chorioamnionitis and FIRS than in those without inflammation ($p<0.001$); and 3) No differences in mRNA expression of NALP3 were detected between the two groups.

CONCLUSIONS: Maternal and fetal inflammation are associated with changes in mRNA expression of major inflammasome components. These findings provide *in vivo* evidence that the inflammasome is involved in the fetal and maternal host response in preterm labor.

13

Transcriptome Characterization in Human Fetuses with Systemic Inflammatory Response. Sally A Madsen-Bouterse,[1] Roberto Romero,[*1] Adi Tarca,[1,3] Jimmy Espinoza,[1,3] Juan Pedro Kusanovic,[1] Ricardo Gomez,[4] Sorin Draghici.[2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Dept of Computer Science, Wayne State Univ, Detroit, MI, USA; [3]Dept of Obstetrics & Gynecology, Wayne State Univ, Detroit, MI, USA; [4]Sotero Del Rio Hospital, Pontificia Universidad Catolica, Santiago, Chile.*

Objective: Delineation of stereotypic changes in leukocyte gene expression is critical for improving the identification and treatment of systemic inflammatory response syndrome (SIRS), a major cause of morbidity and mortality worldwide. A genome-wide expression profile of SIRS was recently attempted by studying the effects of *in vivo* endotoxin challenge in human volunteers (*Nature* 2005, 437:1032). We pursued mRNA profiling in fetuses with proven SIRS to evaluate differences and similarities between adult and fetal immune responses.

Methods: Leukocyte RNA was isolated from cord blood collected into PAXgene RNA tubes from preterm neonates with SIRS (n=10; funisitis, plasma IL-6>19 pg/ml) versus those with no evidence of inflammation (n=7; no funisitis, IL-6<10 pg/ml). Total RNA was globin reduced, amplified, and biotin labeled for hybridization to Illumina Sentrix-Human 6 Expression BeadChips. Differential expression was inferred using moderated t-tests with FDR adjustment of *P*-values. Enrichment analysis of gene ontology and pathways was performed using Onto-Tools and MetaCore (from GeneGo Inc.), respectively.

Results: 1) Abundance of 296 well annotated genes was increased in leukocytes from neonates with SIRS, whereas 252 were decreased ($P \leq 0.05$); 2) Gene ontology analysis showed significant enrichment for: antigen processing and presentation, anti-apoptosis, immune response, and processes critical to cellular metabolism. Molecular functions enriched included signal transduction and transferase activity (e.g., protein-tyrosine kinase activity); 3) Pathway analysis revealed enrichment for pathways key to G-protein coupled receptor signaling, immune response, and metabolism; 4) Of interest, only 48% of altered genes in fetal SIRS were also observed in adults.

Conclusion: This study provides the first genome-wide expression profiling of SIRS in the human fetus. The transcriptome of fetal SIRS differed from that described in experimental models of adult SIRS, which may be attributed to immune maturity and differential composition. Our findings have potential clinical relevance for designing strategies aimed at modulating fetal inflammation.

14

Inflammatory and Thrombotic Responses to Microbial Products in Placental Vessels Are Mediated through Divergent Toll-Like Receptor (TLR) Signaling Pathways: Implications in Fetal Inflammatory Response Syndrome (FIRS). Shekar Davarya, Yuehong Ma, Seth Guller.[*] *OB/GYN and Reproductive Sciences, Yale University, New Haven, CT, USA.*

INTRODUCTION: Poor fetal outcomes including FIRS and neonatal encephalopathy are associated with specific placental lesions. These include thrombotic vasculopathy and neutrophil-associated umbilical vessel inflammation, or funisitis. TLRs participate in cell-specific innate immune responses to pathogen-specific ligands. The role of TLRs in human placental vessel function remains largely unexplored. Our goal was to examine the function of TLR signaling in thrombotic and inflammatory responses using human umbilical vein endothelial cells (HUVECs) as a model. Cells were stimulated with the following TLR ligands: bacterial lipopolysaccharide (LPS, TLR-4), bacterial peptidoglycan (PG, TLR-2), and the viral analog poly(I-C) (PIC, TLR-3). Tissue factor (TF) and interleukin-8 (IL-8) expression were measured as they are the key mediators of thrombosis and neutrophil chemotaxis, respectively.

METHODS: Immunohistochemistry (IHC) was used to localize TLRs in human term placenta (n=3). HUVECs were treated for 48 h with 1μg/ml each of LPS, PIC, or PG in either serum-free medium (SFM) or in medium containing 10% stripped FBS (FBS medium). IL-8 levels in conditioned media and TF levels in cell lysates were measured by ELISA and normalized to total protein content.

RESULTS: IHC revealed TLR-2, -3, and -4 localization to endothelial cells of stem villi in human placenta. For HUVECs maintained in FBS medium, LPS and PIC treatment stimulated IL-8 expression 4- and 80-fold, respectively ($p<0.01$, and $P<0.001$). Similarly, LPS and PIC treatments promoted an 11-fold increase in IL-8 expression in SFM ($p<0.01$). In contrast, PG treatment has no effect on IL-8 levels using SFM or FBS medium. Of note, in FBS medium, TF expression was increased 8- and 3-fold by PG and PIC treatment, respectively. LPS treatment did not significantly affect TF expression.

CONCLUSIONS: Differential patterns of TLR-mediated regulation of TF and IL-8 expression in HUVECs were noted: 1) TLR-3 and -4 signaling increased IL-8 expression; 2) TLR-2 and -3 signaling principally regulated TF expression. These results suggest that microbial-driven large vessel inflammation and neutrophilic infiltration is controlled by TLR-3 and -4, whereas thrombotic events are regulated by TLR-2 and -3. Thus, TLR-specific signaling events may contribute to adverse outcomes in FIRS.

15

Toll-Like Receptor 2 Protein Expression Is Transcriptionally Upregulated during Labor at Term. Refaat E Youssef, Shrikant S Bollapragada, Anne Young, Scott M Nelson, Jane E Norman.[*] *Division of Developmental Medicine, University of Glasgow, Glasgow, Scotland, United Kingdom.*

Background: Increasing evidence indicates that inflammatory mediators play a crucial role in initiation of labor. Toll-like receptors (TLRs) are activated by microorganisms and endogenous ligands with induction of pro-inflammatory cytokines and chemokines expression. We previously demonstrated upregulation of TLR2 and TLR4 mRNA expression in human laboring myometrium (J Soc Gynecol Investig Vol. 13, No. 2 (Supplement), 2006).

Aims: To quantify Toll-like receptor 2 and 4 protein expression in human laboring and non-laboring myometrium

Method: Myometrial samples were collected from pregnant women at time of cesarean section from 4 groups of women: at term before labor (N=9), in labor (N=9), preterm (24 – 36 weeks) before labor (N=9) or in labor (N=6). Samples were divided, snap frozen in liquid nitrogen for protein extraction and formalin preserved, wax embedded for immunohistological staining. TLR2 and TLR4 protein quantification was performed by Western blotting.

Results: As previously demonstrated with mRNA expression, TLR2 protein expression was significantly increased in laboring myometrium while TLR4 protein expression was similar in laboring and in non-laboring myometrium. However, in contrast to our previous mRNA data, we could not demonstrate statistically significant differences in TLR2 and 4 protein expression in term versus preterm myometrium (see tables)

Conclusion: TLR2 plays an important role in myometrial activation during human labor as demonstrated by increased mRNA and protein expression. A complex regulatory process involving mRNA stability, translation, protein modification, and protein half-life can possibly alter the overall protein expression and significance of genetic upregulation. Further studies on functional significance of TLRs activation using LPS and HSP70 as possible ligands are in progress.

TLR2 & 4 protein expression in relation to labor

	In labor		Not in labor		
	Median	IQ range	Median	IQ range	P value
TLR2	15.17	12.00 - 30.73	7.69	3.23 - 10.94	0.002
TLR4	0.25	0.034 - 14.06	0.26	0.018 - 8.21	0.61

Table 1

TLR2 & 4 protein expression in relation to gestation

	Term		Preterm		
	Median	I Q Range	Median	I Q Range	P value
TLR2	11.44	3.27 - 18.35	10.91	6.45 - 17.73	0.71
TLR4	0.045	0.009 - 1.23	2.27	0.004 - 8.93	0.39

Table2

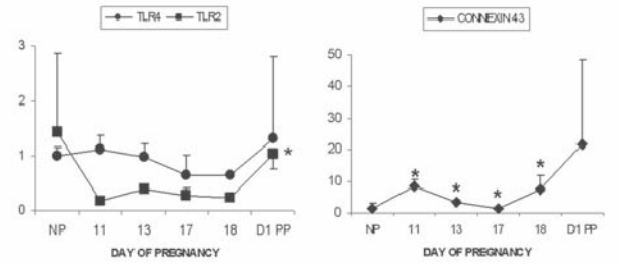
16

Expression of Toll-Like Receptor (TLR)-2 and TLR-4 in Mouse Uterus over the Course of Gestation. Vladimir Ilievski,[1] Shi-Jiang Lu,[1,2] Emmet Hirsch.[*1,2] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

OBJECTIVE: Cells of the innate immune system are found at the maternal-fetal interface. The innate immune system responds to pathogens via specialized membrane-bound proteins known as toll-like receptors (TLR). TLR-2 mediates cellular responses to Gram positive organisms via their membrane lipoproteins, glycolipids and peptidoglycans. TLR-4 recognizes Gram negative organisms by binding lipopolysaccharide (LPS). This study was conducted to characterize the presence and regulation of TLR-2 and TLR-4 in mouse uterus over the course of gestation.

METHODS: Total RNA was extracted from the uteri of non pregnant and pregnant CD-1 mice at gestational days 11, 13, 17, 18 and postpartum. Real-time PCR using ABI TaqMan reagents and normalized to the expression of GAPDH (a housekeeping gene) was conducted for TLR-2, TLR-4 and connexin 43 (a gap junction protein that undergoes increased expression in mice beginning around 18 days of pregnancy, used as a positive control). Three to seven mice were studied at each time-point. Student t tests were used and the p value for significance was set at 0.05.

RESULTS: TLR-4 mRNA levels did not change over the course of gestation. TLR-2 mRNA levels were higher in non-pregnant and postpartum mice than in pregnant mice. Cx43 mRNA increased one day prior to delivery, as expected.



CONCLUSION: Uterine TLR-4 mRNA levels are stable over gestation in the mouse, while TLR-2 levels are suppressed during pregnancy. This pattern may serve a protective function by decreasing sensitivity to TLR-2 signals over gestation.

Supported by RO1HD41689.

17

The Role of Toll-Like Receptor 2 (TLR-2) Activation in Bacterially Induced Preterm Labor in Mice. Vladimir Ilievski,[1] Emmet Hirsch.[*1,2] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

OBJECTIVE: Toll-like receptors (TLRs) are membrane-bound proteins that recognize structural components of bacterial and viral pathogens and initiate host defense responses. TLR-2 is implicated in the recognition of Gram positive bacteria such as group B β-hemolytic streptococcus (GBBS) via their cellular constituents, including peptidoglycan (PGN). The objective of this project was to investigate *in vivo* the role of TLR-2 in bacterially-induced and inflammation-induced labor.

METHODS: Inbred TLR2-deficient mice ($Tlr2^{tm1Kir}$) and control wild type mice (C57BL/6J) on day 14.5 of pregnancy were given intrauterine injections of PGN, killed GBBS or killed *E. coli* (a Gram negative bacterium). Preterm delivery (the finding of at least one fetus in the cage or lower vagina within 48 hours of surgery) was recorded.

RESULTS:

Number (%) of pregnant dams delivering <48 h after treatment

Treatment (per mouse)	*E. coli* (10^8 organisms)	GBBS (10^8 organisms)	PGN (0.25 mg)
Wild-type	4/4 (100%)	6/10 (60%)	10/15 (67%)
TLR-2 knockout	7/8 (88%)	1/13 (8%)	3/13 (23%)
P value	1.00	0.019	0.03

CONCLUSION: *E. coli*, GBBS and PGN can all induce preterm birth in wild-type mice. Preterm delivery induced by GBBS and PGN, but not *E. coli*, is dependent on TLR-2.

Supported by RO1HD41689.

18

Synergistic Effect of Peptidoglycan, a Ligand for Toll-Like Receptor (TLR)-2, and Polyinosinic-Polycytidylic Acid, a Ligand for TLR-3, on Expression of TLR-Dependent Genes. Vladimir Ilievski,[1] Emmet Hirsch.[*1,2] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

OBJECTIVE: Toll-like receptors (TLRs) are membrane-bound proteins that recognize structural components of bacterial and viral pathogens and initiate host defense responses. TLR-2 mediates cellular responses to gram-positive organisms. Peptidoglycan (PGN), a major component of Gram-positive bacterial cell walls, is a prototypical TLR-2 ligand. TLR-2 induces the early expression of inflammatory cytokines via activation of NF-κB. TLR-3 is involved in the response to viral infections by recognizing double-stranded RNA and is responsible for induction of interferon β and interferon-dependent gene products as well as a late-phase NF-κB response. Polyinosinic-polycytidylic acid (poly(I:C)) is a TLR-3 ligand. The objective of this project is to examine the effect of mimicking combined Gram-positive and viral infection (superinfection) on cellular responses.

METHODS: The mouse macrophage-like cell line RAW 264.7 was obtained from ATCC and cultured in DMEM with 10% fetal bovine serum, 1% streptomycin and 1% penicillin. Total cellular RNA was isolated after 5 hours of treatment with either PGN (1 μg/ml), poly(I:C) (10 μg /ml) or both PGN and poly(I:C). Multiplex RT-PCR was performed using ABI TaqMan reagents for inducible nitric oxide synthase (iNOS), interleukin 1-beta (IL-1β), interferon-beta (IFNβ), tumor necrosis factor-alpha (TNFα) and the chemokine RANTES (CCL5).

RESULTS: Co-stimulation of cells with both PGN and poly(I:C) resulted in synergistic expression of iNOS, IL-1β, TNFα and CCL5 ($p<0.05$ for all), but not IFNβ, compared with either PGN or poly(I:C) alone. Both PGN and poly(I:C) induce the expression of TLR-2, but not TLR-3, above background levels.

CONCLUSION: Compared with PGN or poly(I:C) alone, simultaneous exposure of cells to both PGN and poly(I:C) exerts a synergistic effect on the expression of iNOS, IL-1α, TNFα and CCL5, but not IFNβ. The mechanism for this synergy may involve a positive feedback loop of induction of TLR-2 following TLR-3 activation. This finding may have implications for superimposed bacterial and viral infections.

Supported by RO1HD41689.

19

Resistance to *E. coli*-Induced Preterm Labor in Mice Deficient in IL-1 and TNF Signaling Does Not Depend on Altered Regulation of Uterine iNOS, IL-1β, IFNβ or TNF. Yana Filipovich,[1] Emmet Hirsch.[*1,2] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

OBJECTIVE: We have shown previously using mutant mouse strains that the combination of interleukin (IL-) 1 and tumor necrosis factor (TNF) signaling is essential for a normal response to bacterially induced preterm delivery, while IL-1 signaling alone is not. In the present study we explore mechanisms underlying this observation.

METHODS: Mice doubly deficient in the type I receptors for IL-1 and TNF (ILIRI/Tnfrsf1a DKO) and wild-type controls (WT) received either 7 x 10^7 heat-killed *E. coli* organisms or sterile medium by intrauterine injection at 14.5

days of pregnancy (3-5 animals per treatment per genotype). This inoculum of bacteria was previously determined to cause preterm delivery in 69% of WT and 8% of DKO mice ($p = 0.002$). Total RNA was extracted from uterine tissue harvested 4 and 8 hours after treatment. Relative quantitative PCR was performed for inducible nitric oxide synthase (iNOS), IL-1β, interferon β (IFNβ) and TNF, normalized to the housekeeping gene glyceraldehyde 3-phospate dehydrogenase (GAPDH).

RESULTS: *E. coli* exposure induced increased expression of uterine iNOS, IL-1β and TNF 4 to 8 hours after intrauterine injection, but no differences were observed between mutant and wild-type animals despite the large difference in their susceptibility to bacterially induced delivery. IFNβ expression was not influenced by treatment or genotype.

CONCLUSION: These results demonstrate that although deletion of the type I receptors for both IL-1 and TNF has a profound effect on bacterially induced labor, there is no such effect on the induction of iNOS, IL-1β, TNF and IFNβ mRNA in uterine tissues. The expression of these genes occurs independently of IL-1 and TNF signaling, and they are unlikely to play an essential role in *E. coli* induced preterm labor.

20

The Inflammatory Response of Human Fetal Membrane to Microbial Invasion by *Fusobacterium nucleatum*. MS Hamilton,[1] JS Kroll,[3] AD Edwards,[2] PR Langford,[3] MHF Sullivan.[1] (SPON: Phillip R Bennett). *[1]IRDB; [2]Department of Imaging Sciences; [3]Department of Paediatrics, Imperial College London, London, United Kingdom.*

Objective: Intrauterine infection is a major cause of preterm birth, where the presence of bacterial pathogens can initiate labour by stimulating a maternal inflammatory response. *Fusobacterium nucleatum*, an important oral pathogen, has been closely linked with cases of preterm birth and new evidence has shown haematogenous spread of this pathogen from oral to intrauterine tissues. Here, we explore the inflammatory response of human fetal membranes to live cultures of *F. nucleatum* and bacterial products.

Methods: The optimal conditions for growth of *F. nucleatum* were compared on a range of media, and under anaerobic and low (3%) 0_2 at 37 ºC. Fetal membrane explants were obtained from term pregnant women immediately following elective Cesarean section and allowed to recover in serum-free medium overnight. The explants were incubated with LPS, total cell sonicates or live cultures of *F. nucleatum* and incubated under low and high (19%) 0_2 conditions for 24h. Levels of IL-6 and MMP production in supernatants were compared by ELISA and zymography.

Results: Optimal growth of *F. nucleatum* occurred in BHI medium under anaerobic conditions as well as under the low 0_2 tension found in the fetal membrane during gestation. IL-6 levels were shown to increase upon addition of total cell sonicates (1mg/ml) or LPS (5ng/ml) to fetal membrane explants after 16 and 24h of incubation under low and high 0_2. Similarly, live cultures of *F. nucleatum* resulted in increased IL-6 at inoculums as low as 10^3 CFU/ml. The inflammatory response was more pronounced at the later time points (16 and 24h) and 0_2 levels were observed to affect basal IL-6 levels. MMP-2 and -9 activity were higher in term in labour patients compared to term not in labour and in explants stimulated with *F. nucleatum* bacterial products for 24h.

Conclusions: A more comprehensive understanding of the bacteriology of clinically relevant pathogens during intrauterine infection is critical in reducing preterm birth, as current antibiotic treatments are ineffective. Our results confirm that the presence of *F. nucleatum* significantly enhances the inflammatory response in human fetal membranes under the low 0_2 tension found in fetal membrane during gestation. SELDI mass spectrometry will potentially provide a protein signature whereby markers of intrauterine infection by *F. nucleatum* can be identified.

21

P2Y14/GPR105, a G Protein-Coupled Receptor for UDP-Glucose, Is Involved in the Up-Regulation of Interleukin-8 in Endometrial Cells: Implications for Endometrial Immune System. Toru Arase,[1] Tetsuo Maruyama,[1] Hiroshi Uchida,[1] Takashi Kajitani,[1] Masanori Ono,[1] Takashi Nagashima,[1] Kuniaki Ohta,[2] Maki Kagami,[1] Hirotaka Masuda,[1] Hironori Asada,[1] Yasunori Yoshimura.[*1] *[1]Obstetrics and Gynecology, Keio University, Tokyo, Japan; [2]Obstetrics and Gynecology, Toho University, Tokyo, Japan.*

BACKGROUND : To prevent invasion of pathogens, female genital tract has immunological defense systems, including the acidity of the vagina and immunoglobulin production from the cervix. Also, endometrial epithelial cells function as a barrier against pathogens in assistance with endometrial cytokines and immune cells. Recently, it has been reported that the stimulation of P2Y14/GPR105 (P2Y14) by UDP-glucose (UDP-G) enhances a neutrophil chemoattractant, interleukin-8 (IL-8) production in human immune cells and airway epithelial cells. The purpose of this study is to investigate whether UDP-G regulates the expression of IL-8 via P2Y14 in human endometrium.

MATERIALS AND METHODS: We obtained endometrial tissues from the surgical specimens of consenting reproductive-aged patients without any endometrial disorders or with endometritis. The spatiotemporal expression of endometrial P2Y14 was analyzed by RT-PCR and immunohistochemistry. For in vitro experiments, Ishikawa cells, a human endometrial epithelial cell line, were treated with UDP-G and then subjected to RT-PCR analysis on the expression of IL-8 mRNA.

RESULTS: P2Y14 was exclusively and constantly expressed in the glandular and luminal epithelium of normal endometrium throughout the menstrual cycle, while endometrial tissues with endometritis prominently expressed P2Y14 mRNA as compared to the normal endometrium. Treatment with UDP-G induced the expression of IL-8 mRNA in Ishikawa cells in a dose- and time-dependent fashion.

CONCLUSIONS: UDP-G is an endogeneous molecule and released into the extracellular environment in a lytic manner after cell damage caused by bacterial infection. We here demonstrated that P2Y14 was exclusively and constantly expressed in endometrial epithelium throughout the menstrual cycle but up-regulated by endometritis and it mediated IL-8 induction upon UDP-G stimulation. Taken together, these results support a possible mechanism by which UDP-G released from endometrial cells damaged by infection stimulates IL-8 production via up-regulated P2Y14 in endometrial glandular epithelium, which, in turn, may recruit neutrophils and macrophages thereby preventing the progression of infection.

22

C-Reactive Protein Concentrations Measured Early and Late in Pregnancy Related to Spontaneous Preterm Birth. Janet M Catov,[1,4] Lisa M Bodnar,[1,2,4] Stacy Barron,[3] James M Roberts.[*1,2,4] *[1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Ob/Gyn & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [3]Biology, Emory University, Atlanta, GA, USA; [4]Magee-Womens Research Institute, Pittsburgh, PA, USA.*

Objective: There is evidence that C-reactive protein (CRP), a marker of acute and chronic inflammation, is elevated in early pregnancy among women with preterm birth (PTB). We tested the hypothesis that women with PTB may have an inflammatory phenotype detectable in early pregnancy that persists through delivery by relating early (<21 weeks) and late pregnancy (delivery) CRP concentrations to risk of spontaneous PTB.

Methods: CRP was measured via a high sensitivity ELISA at <21 weeks and at admission for delivery. Women with spontaneous PTB <34 weeks (n=32) or 34-<37 weeks (n=77) were compared to women who delivered ≥ 37 weeks (n=229). CRP was categorized as high <21 weeks (≥8 µg/ml), and high at delivery (≥12 µg/ml). Multinomial logistic models were utilized to relate high CRP early vs. late in gestation to PTB risk, independent of confounders.

Results: Median CRP concentrations <21 weeks were not different according to PTB status (p=0.75). However, women with PTB were more likely to have high CRP <21 weeks (>8 µg/ml) compared to women with term births (<34 weeks, 27.3%; 34-<37 weeks, 23.8%; term, 13.6%; p=0.03). After adjustment, women with both early and late high CRP had a 5.5-fold increased risk for PTB <34 weeks. In contrast, only elevated CRP before 21 weeks was associated with increased risk for PTB 34-<37 weeks. Women with high CRP only late in pregnancy had no increased risk for PTB, and there was no effect modification by race.

Conclusion: High CRP before 21 weeks was associated with increased risk of spontaneous PTB. Our findings provide evidence of an inflammatory phenotype, as measured by CRP, which is detectable early in pregnancy, persists through delivery, and may be more severe in women with early PTB.

High CRP in early (<21 weeks) and late pregnancy (delivery) related to spontaneous PTB risk

High CRP*	N	PTB 34-<37 weeks		PTB <34 weeks	
		%	Adj OR **	%	Adj OR **
Early (-), Late (-)	235	20.4	1.0	7.7	1.0
Early (-), Late (+)	45	24.4	1.2 (0.5-2.7)	11.1	1.6 (0.5-4.8)
Early (+), Late (-)	36	33.3	2.8 (1.2-6.8)	13.9	3.2 (1.0-10.5)
Early (+), Late (+)	22	27.3	2.5 (0.8-7.5)	18.2	5.5 (1.4-21.6)

* High CRP early >8 µg/ml; high CRP late >12 µg/ml; **Adjusted for race, BMI, education, and smoking

23

Inflammation, the Cervix, and Progestational Agents: Unraveling Mechanisms. Hua Xu, Juan Gonzalez, Traci Lifsted, Michal A Elovitz.* *OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Introduction: Recent studies have demonstrated that progestational agents (PAs) can prevent recurrent preterm birth. However, the mechanisms by which PAs exert this effect remain unclear. In an animal model, we have demonstrated that PAs can modify the immune response in the lower genital tract and PAs prevent inflammation-induced preterm birth. These studies were performed to determine the possible mechanisms by which PAs may inhibit the immune response in cervical tissues.

Methods: To mimic the physiological make up of the cervix, a macrophage and epithelial cell co-culture model was established: human cervical epithelial (Hela) cells and PMA-induced human macrophage (U937) cells at epithelial/macrophage cell ratio of 5:1 was created. To look for the responsive receptors to lipopolysaccharide (LPS) and progestational agents, the expression of toll-like receptor (TLR-4), glucocorticoid receptor (GR), progesterone receptor (PR) and androgen receptor (AR) were examined in Hela, U937 and PMA-treated U937 cells by Western blotting. The ability of LPS to stimulate an inflammatory response was assessed by measuring cytokines IL-1β, IL-6 and IL8 by ELISA. To examine the effects of PA on this response, cells were pretreated with medroxyprogesterone (MPA), progesterone (P) or Dexamethasone (DEX) prior to LPS-stimulation. Cytokine production between the treatment groups were compared using One-way ANOVA.

Results: TLR-4, GR, PR and AR were all expressed in HELA cells. AR and PR were not expressed in U937 cells but were expressed in PMA-treated U937 cells. IL-6 was expressed at high levels from co-culture and was unaffected by LPS stimulation. IL-8 was not significantly increased by LPS. IL-1β was increased over 6-fold in response to LPS stimulation ($P<0.001$). All PAs abrograted the LPS-induced IL-1 β production ($P<0.001$) as well as significantly inhibiting baseline production of IL-6 ($P<0.001$). The inhbitory response was equal among the MPA, DEX and P.

Conclusions: With the expression of TLR-4, cervical epithelial cells and macrophages have the ability to iniate an immunne response to bacteria or inflammatory challenge in the lower genital tract. Both cervical epithelial cells and macrophages possess receptors that would allow a response to the diverse biochemical actions of various PA. The modulation of the immune response by PA in this co-culture suggests that PA may prevent preterm birth by targeting immune response in the cervix.

24

Intra-Amniotic Inflammation (IAI) Induces Damage to DNA Present in Amniotic Cavity. Irina Buhimschi,[1] Guomao Zhao,[1] Edmund Funai,*[1] George R Saade,[2] Vineet Bhandari,[1] Catalin Buhimschi.[1] *[1]Ob./Gyn.&Reprod. Sci, Yale University, New Haven, CT, USA; [2]Ob./Gyn.&Reprod. Sci, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: Inflammation induces oxidative injury leading to genomic instability. Our objective was to test the hypothesis that IAI induces damage to amniotic fluid (AF) DNA as reflected by accumulation in AF of 8-oxo-hydroguanine (8-oxoG), a hallmark of hydroxyl radical-induced DNA injury.

Methods: AF was obtained by amniocentesis in 62 women (GA: 27.4±0.5 wks) with preterm labor (n=29) or PPROM (n=33). DNA was extracted from 0.5mL AF enriched in AF cells by non-chaotropic techniques to minimize artefactual oxidation, followed by digestion and analysis by HPLC with dual electrochemical (400mV)/UV detection to quantify 8-oxoG and 2dG (deoxyguanine, the parent DNA base), respectively. The number of modified G bases per 10^5 total dG (8-oxoG/2dG ratio) reflects the extent of DNA damage. A proteomic fingerprint [Mass restricted (MR) score] characteristic for IAI was generated using SELDI-TOF. The MR score ranges from 0-4 (none to all 4 biomarkers present). A score of 3-4 indicates the presence of IAI, while a score 0-2 excludes it. AF IL-6 was measured by ELISA. Placental histology was assessed for evidence of acute inflammation. **Results: 1)** Women with IAI (n=30) were of a significant earlier GA at amniocentesis ($p=0.02$) and delivery ($p=0.002$), had significantly higher AF neutrophil counts ($p<0.001$), IL-6 levels ($p<0.001$), had higher incidence of positive AF cultures ($p<0.001$) and histological chorioamnionitis ($p<0.001$), compared with women without inflammation; **2)** A higher percentage of women without IAI had 8-oxoG levels below the limit of detection compared to those without IAI (MR=3-4: 9% vs. MR=0-2: 30%, $p<0.001$); **3)** Women with IAI had higher 8-oxoG/2dG ratios than women without IAI (median [range], MR=3-4: 2.6 [0-9.2] vs MR=0-2 (0 [0-10.1]), suggesting a higher level of DNA damage in relationship to IAI; **4)** Multivariate linear regression analysis showed that the 8-oxoG/2dG ratio was dependent on the severity of IAI ($p<0.001$), but not on maternal age ($p=0.22$), GA at amniocentesis ($p=0.87$), status of the membranes ($p=0.57$) or AF neutrophil count ($p=0.91$). **Conclusion:** IAI is associated with extensive damage the DNA in AF. Our findings may provide a potential explanation for the short and long-term adverse outcomes reported in the neonates delivered prematurely in the context of intrauterine inflammation.

25

Term Labor Is Characterized by an Inflammatory Pathway Common to Human Fetal Membranes, Myometrium and Cervix. Shrikant S Bollapragada, Refaat Youssef, Fiona Jordan, Anne Young, Ian Greer,* Jane E Norman,* Scott M Nelson. *Reproductive and Maternal Medicine, Glasgow Royal Infirmary, University of Glasgow, Glasgow, United Kingdom.*

Objective: Accumulating evidence suggests that labor is an inflammatory process. Although inflammatory markers are increased with onset of labor, the full extent of the involvement of inflammation has not been established. It is now possible to study this by using a genomic approach. Our aim was to study the differential expression of genes common to the three main maternal tissue types within the uterus (myometrium, cervix and membranes) with onset of labour.

Methods: Gene arrays (Affymetrix v2 U133+2) were carried out on myometrial and cervical biopsies obtained from women undergoing cesarean section at term before labor (**TnL**; n=9) and in spontaneous labor (**TL**; n=9). Analysis was performed using FunAlyse automated pipeline. The data were subjected to low-level analysis using the Robust Multichip Analysis method. Differentially expressed genes were identified by RankProducts. 224 genes were previously identified as differentially expressed in membranes with the onset of labour (Haddad et al, Am J Obstet Gynecol. 2006;195(2):394.e1-24). We compared these against the myometrial and cervical array data.

Results: Of the 224 genes differentially expressed by labor in membranes, 122 were expressed by myometrium and 140 by cervix. There was a strong correlation between the genes expressed by the three tissues: membranes v myometrium ($r=0.59$, $p<0.001$), membranes v cervix ($r=0.54$, $p<0.001$) and myometrium v cervix ($r= 0.59$, $p<0.001$). 99 genes were common to all 3 tissues, however only 67 were consistently up-regulated and 17 consistently down-regulated. The correlation remained strong for all the up regulated genes; membranes v myometrium ($r=0.41$, $p<0.001$), membranes v cervix ($r=0.44$, $p<0.001$) and myometrium v cervix ($r= 0.52$, $p<0.001$), but not for the down regulated ones. In the Ingenuity pathway analysis for the up regulated genes, pro-inflammatory pathways including IL6 signaling and Toll-like receptor signaling pathway featured prominently. "Cell movement" was the most common gene ontology group. We have validated candidate genes for each of these pathways; IL-6, IL-8, TLR 2 and 4 using Northern blot, RT-PCR or ICC.

Conclusion: Genes associated with inflammation and induction of chemotaxis demonstrated the greatest fold change in all three tissues and therefore labor appears to involve "a common inflammatory pathway".

26

Mechanisms of Leukocyte Accumulation and Activation in Chorioamnionitis: Interleukin-1beta and Tumor Necrosis Factor-alpha Enhance Granulocyte Macrophage-Colony Stimulating Factor Expression in Term Decidua. Felice Arcuri,[1] Paolo Toti,[1] Lynn Buchwalder,[2] Marcella Cintorino,[1] Alessandra Casciaro,[1] Frederick Schatz,*[2] Charles J Lockwood.*[2] *[1]Department of Human Pathology and Oncology, University of Siena, Siena, Italy; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA.*

Objective: To compare by immunohistochemistry (IHC) granulocyte macrophage-colony stimulating factor (GM-CSF) expression in the decidua of patients with chorioamnionitis (CA) and gestational age-matched controls, and to test the effects of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1beta) on GM-CSF expression in cultured term decidual cells.

Methods: Immunoreactive GM-CSF levels in decidua were assessed by IHC. Confluent leukocyte-free decidual cells were primed with 10(-8) M estradiol (E2) or E2+10(-7) M medroxyprogesterone acetate (MPA) to mimic the steroidal milieu of pregnancy, then switched to a defined medium with corresponding steroid(s) +/- TNF-alpha or IL-1beta. After 24 h, secreted GM-CSF levels were assessed by ELISA with the results normalized to cell protein, and GM-CSF mRNA levels were measured by quantitative RT-PCR.

Results: Increased GM-CSF IHC levels were observed in decidua from pregnancies complicated by CA compared with controls. Levels of GM-CSF in E2+MPA treated cultures increased respectively of 18 fold +/- 6.7 (mean +/- SEM; $p<0.05$) and 245 fold +/- 33.8 ($p<0.05$) after addition of TNF-alpha and IL-1beta at 1.0 ng/ml each (n = 8). When compared with incubations with E2 alone, E2 plus MPA neither significantly affected GM-CSF levels nor altered the response to the cytokines. The induction of GM-CSF protein by TNF-alpha and IL-1beta was concentration-dependent with maximum effects for both cytokines at 10 ng/ml. The ELISA results were confirmed by quantitative RT-PCR that demonstrated parallel changes in mRNA levels.
Conclusions: This study reveals that GM-CSF, a potent and specific leukocyte chemoattractant and activator, is strongly expressed in term decidua from patients with CA. Our observation that TNF-alpha and IL-1beta greatly enhance GM-CSF expression in term decidual cells suggests that these cytokines are important regulators of CA-related decidual leukocyte infiltration and activation.

27

Expression of Triggering Receptors of Myeloid Cell (TREM-1) in Human Fetal Membranes. Ramkumar Menon, Salvatore J Lombardi, Stephen J Fortunato. (SPON: Kelle H Moley). *The Pernatal Research Center, Nashville, TN, USA.*
OBJECTIVE: Triggering Receptors of Myeloid Cell (TREM-1) are transmembrane glycoproteins that trigger phagocyte secretion of proinflammatory cytokines initiating the innate immune response. The inflammatory response is exaggerated by TREM1 in response to bacterial cell wall products such as lipopolysaccharide (LPS) and lipoteichoic acids. Soluble forms of TREM1 indicate inflammatory response. This study documents TREM1 expression in fetal membranes and its significance in response to *in vitro* stimulation of fetal membranes by LPS.
METHODS: Amniochorionic membranes (n = 25) collected from women at term with no pregnancy complications, not in labor, undergoing elective repeat Cesareans were placed in an organ explant system. After 48 hours in culture, membranes were stimulated with E.coli LPS (100 ng/ml). Tissues and media samples were collected after a 24 hour stimulation. Fetal membrane expression of TREM1 was studied by RT-PCR using specific primers, and soluble TREM1 (sTREM1) in culture media was measured using ELISA. Mann-Whitney tests were used to analyze statistical significance. Additional membranes were collected from women with documented intraamniotic infection (IAI) delivering preterm and subjected to the analysis outlined above for TREM1.
RESULTS: Amniochorion at term did not express TREM1 in culture; however, it was induced by LPS as documented by RT-PCR. TREM1 expression was also seen in membranes collected from women with preterm birth and IAI. Soluble TREM1 was measurable in culture media even in the absence of documented membrane expression. LPS stimulation significantly increased the release of sTREM1 (median 328; range 64-4136) from amniochorion compared to control (188; range 31-1405; $p = 0.03$). We also examined ethnic differences in TREM1 expression and production and no differences were observed between the two ethnic groups (Caucasians and African-Americans).
CONCLUSIONS: This is the first study to document the expression of TREM1 in intrauterine tissues. As a proinflammatory enhancer, the presence of TREM1 in intrauterine tissue is of considerable significance as bacterial ligands and their recognition molecules (Toll like receptors –TLRs) engage TREMs to boost the inflammatory response by releasing cytokines. Our study documents that fetal membranes respond to an infectious stimuli by expressing TREM1 *in vitro* and similarly to IAI *in vivo* suggesting a prominent role for these molecules in inflammation during pregnancy.

28

Regulation of Chemotaxis and Reactive Oxygen Species Production by Maternal Leukocytes during Parturition. Meifang Yuan,[1] Iain B McInnes,[2] Margaret M Harnett,[2] Jane E Norman.*[1] *[1]Reproductive and Maternal Medicine, Division of Developmental Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Division of Inflammation, Infection and Immunity, University of Glasgow, Glasgow, United Kingdom.*
Background: The maternal inflammatory response is inhibited during human pregnancy. Increasing evidence however suggests that labour is an inflammatory event. We have shown a massive influx of neutrophils into the myometrium in labour. Neutrophils are activated in response to various stimuli, including chemotactic factors, cytokines, and immune complexes to produce superoxide and other oxygen radicals, which may be important causes of tissue damage in a number of inflammatory conditions.
Objective: To test the hypothesis that leukocytes are activated in labour, and that leukocytes from labouring women will show greater chemotaxis and intracellular reactive oxygen species production.
Methods: Human neutrophils were separated from heparinized maternal peripheral blood samples obtained from the following groups of women: 28-34 week gestation preterm not in labour (PTNL), n=10; 37-42 week gestation term not in labour (TNL), n=10; and 37-42 week gestation term in labour (TL), n=10 using dextran and Ficoll-hypaque. Chemotaxis was studied in response to fMLP in a 96-well chemotaxis chamber. Intracellular reactive oxygen species production (ROS) in whole peripheral blood was quantified by measurement of the fluorescent DCF generated from the ROS probe DCFH-DA (dichlorofluorescein diacetate) by flow cytometry. Student's t-test was used for statistical analysis.
Results: Chemotaxis of neutrophils (Table I) and basal ROS values (granulocytes and monocytes) (Table II) were significantly greater in labouring versus non labouring women.
Conclusions: This study confirmed our initial hypothesis that leukocytes are activated in labouring women. The mechanism of leukocyte activation in the process of parturition remains to be further elucidated.

Chemotaxis Assay

	Ratio
PTNL	1.091±0.257
TNL	0.976±0.141
TL	2.798±0.766**

The number of cells migrating towards to fMLP (1uM) were expressed as a ratio of those randomly migrating in the absence fMLP. **P<0.001, as compared with PTNL group.

ROS Production

	MFI (granulocytes)	MFI (monocytes)
PTNL	90.2±14.8	219.8±23.0
TNL	85.7±19.4~	209.5±20.6~
TL	125.2±33.3*	328.7±34.3*

Basal MFI (mean fluorescent intensity) of DCF as assessed by flow cytometry. *P<0.05, compared with PTNL. ~P>0.05, compared with PTNL.

29

Does Maternal and Fetal Response to Intrauterine Inflammation Differ with Gestational Age? Juan Gonzalez, Traci Lifsted, Hua Xu, Michal A Elovitz.* *OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*
Objective: Activation of inflammatory pathways are implicated in preterm birth (PTB). We have previously demonstrated that a high dose of intrauterine lipopolysaccharide (LPS) results in PTB in mice. These studies sought to assess 1) if the maternal response to LPS was gestational age dependent, 2) if lower doses of LPS could evoke PTB, and 3) to determine if exposure to LPS, in the absence of PTB, resulted in altered growth patterns in the offspring.
Methods: Using a mouse model of intrauterine inflammation, CD-1 timed-pregnant mice were randomized to intrauterine infusion of saline (NS) or different doses of LPS (62.5, 125 or 250 micrograms) on E15, 16, 17 or 18 (term is E19). 14 dams received NS and 53 dams were treated with differing doses of LPS. PTB and maternal mortality were assessed at 24 hr intervals until post-natal day 7 (PND7). The number of live pups was recorded and neonatal weights were assessed on PND 7 and PND 20. The reference group for neonatal weights were pups born from dams receiving intrauterine saline.
Results: The highest dose of LPS consistently results in 100% preterm birth on E15 and E16 while the middle dose results in 70-80% PTB rate. The lowest dose of LPS results in a 30-50% PTB rate between E15-17. The highest dose of LPS does not result in any live pups at term when administered on E15, 16 or 17. On E18, all doses of LPS result in some live pups at term. The lower and middle doses of LPS result in incomplete deliveries and maternal death 11-25% of the time regardless of gestational age. In dams with live pups at term, the mean number of live pups per dam was not significantly different between NS-treated (10.4 +/- 2.2) and LPS treated dams (8.9 +/-3.0). On PND7, the mean neonatal weight was significantly decreased in dams treated with low dose LPS on E15 ($p<0.001$) while dams treated with low dose LPS on E18 were significantly larger compared to saline($p=0.002$). On PND20, dams treated with low dose LPS on E16 and E18 were significantly heavier than saline controls ($p=<0.001$).
Conclusions: In CD-1 mice, the maternal response to LPS near term is quite variable suggesting that diverse genetic pathways are involved in the host immune response. In utero exposure to inflammation results in growth distrubances in the offspring that appears to be both dose and gestation age dependent. Whether these growth distrubances are associated with long-term adverse outcomes requires further investigation.

30

Azithromycin (AZI) Dose-Finding Study for Treatment of Ureaplasma Intraamniotic Infection (IAI) in Rhesus Monkeys. Drew W Sadowsky,[*1] Michael D Reed,[2] Mary A O'Riordan,[2] Lynn B Duffy,[3] Kenneth B Waites,[3] Miles J Novy.[*1] *[1]Reprod Sci, Oregon National Primate Res Ctr, Beaverton, OR, USA; [2]Pediatric Pharm, Case Western Reserve Univ, Cleveland, OH, USA; [3]Pathology, Univ Alabama Birmingham, Birmingham, AL, USA.*

Objective. The efficacy of AZI in treatment of ureaplasma IAI has not previously been determined. We characterized the transplacental pharmacokinetics (PK) of AZI in the treatment of IAI with *Ureaplasma parvum* in a nonhuman primate model.

Study Design. Eleven chronically instrumented rhesus monkeys received intraamniotic inoculation of 10^7 cfu *U. parvum* (serovar 1) at 126-142 days of gestation (term=167 days). Six animals received maternal i.v. AZI (12.5mg/kg q12h or q6h for 10 days) after uterine activity increased. Maternal, fetal and amniotic fluid (AF) compartments were serially sampled during AZI dosing and washout. Plasma and AF AZI levels were determined by highly sensitive LC-MS. Non-compartmental PK analyses were compared with quantitative cultures and specific PCR for *U. parvum*.

Results. AF *U. parvum* reached a peak of $3.0 \pm 1.2 \times 10^6$ cfu/ml by 43 ± 5h after inoculation, and stabilized thereafter at $0.5 \pm 0.2 \times 10^6$ cfu/ml. AF *U. parvum* was rapidly reduced to 5% of pre-AZI cfu within 24h and eradicated from AF in all AZI treated animals by 86.8 ± 10h ($P<0.001$ vs untreated). Placental and fetal tissues in 5 of 6 animals were culture and PCR negative after 10 days of AZI treatment. High maternal plasma AZI levels of 818-5973ng/ml were achieved transiently during a single i.v. infusion (n=4) and decayed rapidly to 214 ± 16.9 ng/ml by 2h and 61.5 ± 11.9 ng/ml within 12h. Corresponding fetal plasma AZI was 3.5% (2h) and 16.4% (12h) of maternal levels. Inhibitory concentrations of AZI in AF ranged from 12.5-30% of maternal levels (n=5) early in dosing and rose to 72-236% (n=2) after multiple day therapy indicating accumulation. A slow decay in AF AZI during washout occurs with 40ng/ml remaining even after 12 days, likely due to extended tissue penetration. PK estimates varied among animals and between single and multiple AZI infusions. Estimated $t_{1/2}$ of AZI was 35-43h in maternal plasma, and as long as 99-347h in AF.

Conclusions. Maternally administered AZI distributes and accumulates in AF and eradicates *U. parvum* from AF in 4 days. Short-term therapy rapidly reduces AF cfu, but repeated dosing may be necessary to optimize eradication of *U. parvum* from fetal and placental tissues. Support HD06159, RR00163, HD031323.

31

Azithromycin (AZI) and Antiinflammatory Therapy for Intraamniotic Infection (IAI) with *Ureaplasma parvum* in a Non-Human Primate Model. Drew W Sadowsky,[*1] Lois M Colgin,[1] Lynn B Duffy,[2] Kenneth B Waites,[2] Michael G Gravett,[*3] Miles J Novy.[*1] *[1]Reprod Sci, Oregon National Primate Res Ctr, Beaverton, OR, USA; [2]Pathology, Univ Alabama Birmingham, Birmingham, AL, USA; [3]OB/GYN, Univ Washington, Seattle, WA, USA.*

Objectives. *U. parvum* IAI results in subacute chorioamnionitis, preterm labor (PTL), and fetal pneumonia. The efficacy of maternal AZI treatment for *U. parvum* IAI has not been established. We compared therapeutic effects of AZI alone, or in combination with dexamethasone (DEX) and indomethacin (INDO) because antibiotics plus immunomodulators prolong gestation in monkeys with group B streptococcal IAI (AJOG 189:s56,abstr#3).

Study Design. Eleven chronically instrumented rhesus monkeys received intraamniotic inoculation of 10^7 cfu *U. parvum* (serovar 1) at 130±1.5 days of gestation (mean±SEM, term=167 days). Six animals received maternal i.v. AZI (12.5mg/kg q12h or q6h/10d) either alone (n=3) or in combination (n=3) with DEX (4mg/kg/day iv/4d) and INDO (100mg/day PO/5d) after uterine activity (UA) increased. UA, amniotic fluid (AF) leukocytes, cytokines (IL-1β, IL-6, IL-8, TNF-α), prostaglandins (PGE_2, $PGF_{2\alpha}$), and *U. parvum* quantitative cultures were serially measured. Fetal and gestational tissues were examined histopathologically.

Results. *U. parvum* IAI caused significant increases in AF cytokines, PG's and leukocytes (n=11, $P<0.05$). Untreated animals were delivered by 7.2±2.1 days. Both AZI alone and AZI/DEX/INDO successfully eradicated *U. parvum* from AF by 3.6±0.4 days and prolonged gestation compared to untreated animals (ANOVA, $P<0.05$), but the inoculation-to-delivery interval was not different between AZI alone and AZI/DEX/INDO (19.7±2.6 days, 18.8±2.8 days, respectively). AZI alone reduced AF $PGF_{2\alpha}$ and TNF-α, while combined therapy also reduced AF IL-1β, IL-8, and UA (ANOVA, $P<0.05$). AF proinflammatory mediators rose following cessation of treatment before labor onset at 146-155 days. Both AZI and AZI/DEX/INDO prevented lung damage seen with prolonged exposure to *U. parvum*, but histologic chorioamnionitis persisted.

Conclusions. IAI with *U. parvum* was successfully treated with maternal AZI therapy resulting in clearance of AF and fetal infection, reduction in AF inflammatory mediators, prolongation of gestation, and significant resolution of fetal pneumonia. The antiinflammatory properties of the macrolide AZI may contribute to the therapeutic effect in prolonging pregnancy with *U. parvum* IAI. HD06159, AI42490.

32

Progestational Agent Modulation of Gene Expression of Apoptotic, Toll-Like Receptor (TLR), and Inflammatory Gene Pathways in Lipopolysaccharide (LPS)-Stimulated Human Decidual Cells. Hyagriv N Simhan,[*1,2] Jye-Ping Chiao,[1,2] Steve N Caritis.[*1,2] *[1]Ob/Gyn, Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetric-Fetal Pharmacology Research Units Network, Bethesda, MD, USA.*

Objective: Decidual cells are immunologically active cells in the host response to upper genital tract infection. TLRs play a key role in pathogen recognition and initiation of the immune response, followed by gene expression in apoptotic and inflammatory pathways. Recent trials support progestational agents for the prevention of prematurity, although the mechanism of this effect is unknown. We sought to determine if progesterone (P), 17-α-hydroxyprogesterone (17P) and 17-α hydroxyprogesterone caproate (17PC) exert an anti-inflammatory effect via TLR signaling, downstream inflammation, and apoptotic pathways in the response of human decidua to LPS.

Study Design: Decidual cells from 6 women undergoing cesarean delivery without labor at term were cultured to confluence and incubated under 7 conditions: 1) vehicle for 24h followed by PBS for 24h. 2) P for 24h followed by PBS for 24h. 3) 17P for 24h followed by PBS for 24h. 4) 17PC for 24h followed by PBS for 24h. 5) vehicle for 24h followed by E. coli LPS (25 ng/mL) for 24h. 6)17P for 24h followed by LPS (25 ng/mL) for 24h 7) 17PC for 24h followed by LPS (25 ng/mL) for 24h. RNA was extracted, isolated, and purified and relative expression of 384 genes normalized to GAPDH and β-actin was determined using human PCR Array for apoptosis, inflammatory cytokines and receptors, and TLR signaling pathways (SuperArray Bioscience, Frederick, MD). ANOVA with Bonferroni post-hoc testing was utilized. A conservative α of 0.001 was selected a priori.

Results: Compared with control cells, cells exposed to P, 17P, or 17PC did not demonstrate any significant activation of genes in the pathways of interest. As expected, LPS induced a vigorous response in decidual cells, with broad increases in many genes in the pathways of interest. Pretreatment of cells with P, 17P, or 17PC prior to LPS treatment did not abrogate the gene expression changes in comparison to non-pretreated cells.

Conclusions: The human decidual cell is immunologically potent, exhibiting broad gene expression changes in apoptosis and inflammation pathways. The progestational agents studied did not modulate this response, and thus, immunomodulatory action of these agents on the human decidual cells does not explain their apparent clinical effects.

33

Racial Differences in Cytokine Concentrations Associated with Bacterial Vaginosis. Kelli K Ryckman,[1,2] Scott M Williams,[1,2] Marijane A Krohn,[3] Hyagriv N Simhan.[*3] *[1]Department of Medicine, Vanderbilt University, Nashville, TN, USA; [2]Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; [3]Department of Obstetrics and Gynecology, Magee Womens Research Institute, Pittsburgh, PA, USA.*

Objectives: We examined the association between inflammatory cytokine levels and bacterial vaginosis (BV) in pregnant Caucasian and African-American women. **Methods:** This case-control study assessed cytokine concentrations in 132 Caucasian women; 80 with normal vaginal flora, 17 with intermediate status, 35 with BV and 141 African-American women; 59 who were normal, 21 with intermediate status, 61 with BV. Twenty-eight inflammatory cytokines were assessed by the Luminex® multiplex assay. BV status was determined by Gram-stained vaginal smears and evaluated using the Nugent score. To determine differences between BV status and cytokine concentration for African-Americans and Caucasians one-way and two-way analysis of variance (ANOVA) was performed on cytokine concentration values that have been natural log transformed to address distributions not normally distributed. **Results:** The one-way ANOVA showed that Caucasian women differed significantly by BV status for the following cytokines: interferon gamma (IFN), interleukin-1 alpha (IL-1a), interferon gamma inducible protein 10 (ip10), monocyte chemotatic protein 1 (mcp-1), platelet derived growth factor aa (pdgf-aa), platelet derived growth factor ab/bb (pdgf-aa/bb) and fms-related tyrosine kinase 3 (flt-3) and African-American women differed by BV status

for IL-1a, interleukin-3 (IL-3) and interleukin-6 (IL-6). The two-way ANOVA showed that when race was controlled for IL-1a, interleukin-1 beta (IL-1b), ip10, mcp-1, pdgf-aa and pdgf-aa/bb were significant for BV status. For example in Caucasians the mean log values for IL-1a were 7.46 for BV subjects, 6.41 in intermediates and 6.99 for normal status (P=0.019) and in African-Americans the mean log concentrations were 7.33 for BV subjects, 6.93 for intermediates and 6.42 for normal status (P<0.001). **Conclusions:** These results show that there are differences in inflammatory cytokine concentrations with regards to BV status for many different cytokines. These results support the conclusions that Caucasian and African-African women have different cytokine responses to BV. Such a finding may be important in explaining the role of cervical cytokines in pregnancy outcomes between the two groups.

34

The Effect of Labour and Mechanical Stretch on PBEF, IL-1β and IL-8 Expression in Human Amnion Cells. Aarthi R Mohan,[1] Suren R Sooranna,[1] Peta Grigsby,[2] Leslie Myatt,[*2] Phillip Bennett,[*1] Mark R Johnson.[*1] *[1]Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, London, United Kingdom; [2]Obstetrics and Gynaecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*

Introduction: Human labour involves inflammatory cytokines including IL-1β, IL-8 and pre-B cell colony-enhancing factor (PBEF). Stretch of the uterus plays an important role in labour. We have previously described a biphasic increase in NF-κB activation and COX-2 expression by stretch of amnion cells. We hypothesise that this biphasic effect may be caused in part by the increase in inflammatory cytokines as a result of NF-κB activation. We have also compared expression of IL-1β, IL-8 and PBEF between prelabour, labour, term and preterm amnion samples.

Methods: Amnion tissue samples were taken from women undergoing LSCS either before or after the onset of term or pre-term labour and frozen for extraction of RNA (n=9 for PTNL and PTL; n=8 for TNL and n=10 for L). Confluent primary amnion epithelial cells obtained from women undergoing term elective LSCS (n=7) and were subjected to a static stretch of 11% for up to six hours using a Flexercell strain unit. Unstretched cells were used as controls. At the end of the incubations, RNA was extracted and converted to cDNA. PBEF, IL-1β and IL-8 and GAPDH mRNA were measured by quantitative real-time PCR using a Rotor-Gene™PCR machine.

Results: In the amnion tissue samples, there was a three-fold increase in IL-1β expression in the term labour samples as compared to the term non-labour samples (p=0.058). There was also a significant 10-fold increase in IL-8 expression in the term labour samples as compared to the non-labour samples (p=0.024). There was no increase in PBEF expression in the labour samples compared to the non-labour samples for both preterm and term tissue. There was a marked increase in IL-1β mRNA levels at four and six hour stretch but not at earlier times. There was no increase in IL-8 or PBEF expression at any of the stretch time-points.

Conclusions: Our results show that PBEF may not be important in the stretch-induced increase of NF-κB activation in amnion, or in labour-associated changes, unlike IL-1β or IL-8. It is possible that the first peak of NF-κB activation by stretch we have previously described, causes an increase in IL-1β, which induces a second peak in NF-κB activation at six hours stretch but IL-8 appears not to be responsible for this second peak.

35

Mechanical Stretch of Human Amnion Cells Increases Cyclo-Oxygenase Type 2 and Interleukin-1 Beta Expression Via MAP Kinase Activation. Aarthi R Mohan, Suren R Sooranna, Mark R Johnson,[*] Phillip R Bennett.[*] *Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, London, United Kingdom.*

Introduction: In human parturition at term, both the myometrium and fetal membranes are stretched. An important source of prostaglandins is the amnion. We have previously shown that stretch of the amnion increases COX-2 expression and NF-κB DNA binding in a time-dependent manner. In this study, we have examined the effect of mechanical stretch at various time-points on MAP kinase activation, COX-2 and IL-1β expression and COX-2 protein synthesis.

Methods: Confluent amnion epithelial cells were subjected to a static stretch of 11% for up to six hours using a Flexercell strain unit (Flexcell International Corp., McKeesport, Pa). Unstretched cells were used as controls. Cells were incubated with either 10μM U0126 (an ERK inhibitor) for two hours, 20μM SP600125 (a JNK inhibitor) for one hour or 10μM SB203580 (a p38 inhibitor) for 30 minutes prior to stretch, after which, the medium was removed and the cells were either frozen at -80C for expression studies, or were lysed and the proteins separated. Western analysis was used to measure phospho-ERK (Thr202/Tyr204), phospho-JNK (Thr183/Tyr185), phospho-p38 (Thr180/Tyr182) and COX-2 protein.

Results: Stretch caused a time-dependent increase in COX-2 at both the mRNA and protein levels. Western analysis showed that U0126 (ERK inhibitor) inhibited this increase in COX-2 protein synthesis at both the early and later time-points but SB203580 (p38 inhibitor) decreased COX-2 synthesis at only the later time-points. SP600125 (JNK inhibitor) did not have an effect on COX-2 synthesis at any of the time-points of stretch. U0126 and SB203580 but not SP600125 inhibited COX-2 mRNA expression after four and six hours stretch. Stretch caused a 15-fold increase in IL-1β expression after six hours. This was markedly reduced by all three MAP kinase inhibitors. We have shown that, in the amnion, the main MAP kinses that are present are ERK and p38 with less JNK present.

Conclusions: The increase in COX-2 expression and protein synthesis by stretch in human amnion cells appears to be mediated by ERK and p38 MAP kinases. JNK does not appear to play an important role in this increase. However, all three MAP kinases have been shown to be important in the stretch induced increase in IL-1β expression.

36

Progestin and a MAPK Inhibitor Blunt TNF-a and IL-β Induced Matrix Metalloproteinase 1 and 3 Expression in Term Decidual Cells: Implications for Treatment of Chorioamnionitis-Induced Preterm Delivery. Ceyda Oner, Frederick Schatz,[*] Gulnur Kizilay, Umit Kayisli, Lynn F Buchwalder, Mizanur Rahman, Aydin Arici,[*] Charles J Lockwood.[*] *Obstetrics/Gynecology & Repro. Sci, Yale University, New Haven, CT, USA.*

Objective: Chorioamnionitis (CAM) is associated with elevated amniotic fluid tumor necrosis factor-alpha (TNFa) and interleukin-1beta (IL-1b) levels. We hypothesize that these pro-inflammatory cytokines activate p38 MAPK signaling in decidual cells (DCs) to enhance expression of the matrix metalloproteinases, MMP-1 and MMP-3. These MMPs can promote preterm delivery (PTD) by degrading the extracellular matrix of the decidua, fetal membranes and cervix.

Study design: Immunohistochemistry for MMP-1, MMP-3, the DC marker vimentin and the trophoblast marker cytokeratin in decidua of normal term (n=5) and CAM-complicated (n=5) deliveries was assessed by HSCORE. Confluent term DCs (n=4) were primed with 10^{-8} M estradiol (E_2) or E_2 + 10^{-7} M medroxyprogesterone acetate (MPA) for 7 days, pre-incubated +/- the specific p38 MAPK inhibitor (SB203580) for 30 min then incubated in a defined medium with corresponding steroid(s) +/- IL1β or TNFa. After 24 hours, secreted MMP-1 and MMP-3 levels were measured by ELISAs and Western blotting; mRNA levels were assessed by quantitative RT-PCR.

Results: H scores of MMP-1 and MMP-3 levels in DCs of CAM-complicated decidua (257.5±7.5 and 197.5±20, respectively, were higher than normal decidua (140 ± 10, 140±7, respectively; p<0.05). In cultured DCs incubated with E_2, 1.0 ng/ml of TNFa increased secreted MMP-1 and MMP-3 levels by 19±1 and 9±2-fold, respectively, and IL1b increased them by 16±3 and 20±2-fold, respectively; p<0.05. In parallel incubations with E_2 + MPA, both basal MMP-1 and MMP-3 output were lowered by approximately 75% (p< 0.05) while TNFa- and IL1β-enhanced MMP-1 and MMP-3 levels were blunted by more than half (p<0.05). The p38 MAPK inhibitor also suppressed TNFa and IL1β-induced MMP-1 and MMP-3 secretion. Western blotting confirmed the ELISA results and mRNA levels corresponded to changes in MMP-1 and –3 protein levels.

Conclusions: Involvement of the progesterone receptor and the MAPK signaling pathway in inflammatory cytokine-enhanced MMP-1 and MMP-3 expression in term DCs suggests that exogenous progestins and MAPK inhibitors offer alternative therapeutic approaches in preventing CAM-induced PTD.

37

Polymorphisms in the Promoter Region of the Interleukin-10 (IL-10) Gene in Women with Cervical Insufficiency. Jennifer Warren, Robert M Silver,[*] Lesa Nelson, Jess Dalton, Kristi Nelson. *Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA.*

Objective: The etiology of cervical insufficiency (CI) is unclear but may involve alterations in inflammatory responses. Genotypic variations in the promoter region of the IL-10 gene have been associated with variations in other well characterized, inflammatory-mediated processes. Our objective

was to determine whether polymorphisms in the promoter region of the IL-10 gene are more common in women with CI compared to women without the condition.
Methods: Medical, obstetric, and family histories, and blood were obtained from women with (N=121) and without (N=157) cervical insufficiency. DNA was extracted and purified using Puregene Isolation kits. Samples were analyzed for the IL-10-1082 G/A polymorphism and genotyped for the IL-10.G microsatellite in the promoter region of the IL-10 gene.
Results: The G/A polymorphism at the -1082 position in the promoter region of the IL-10 gene occurred with similar frequency in cases and controls. The GG genotype was found in 17.1% of cases and 23.8% of controls (p=NS). The GA gentoype was present in 45.9% of cases and 45.2% of controls (p=NS). The IL-10.G microsatellite contained 10 alleles (G6-G16). The G13 allele was present more frequently in cases, 23.6%, compared to controls,14.6% ($p<0.05$, Fisher's exact). The other nine alleles were found in similar frequency between cases and controls.
Conclusions: IL-10 is known to down-regulate inflammation. The G13 allele, one of the two most common polymorphisms in this microsatellite, occurred more frequently in our population of women with CI compared to controls, suggesting that alterations in inflammatory-mediated processes may play a role in this disease state.

38

Presence of Infections or Duration of Labor Are Not Associated with Spontaneous Separation of Fetal Membranes. D Kumar,[1] J Smith,[1] C Weber,[1] R Moore,[1] B Stetzer,[2] Brian Mercer,[*2] J Mansour,[3] John Moore.[*1,2] *[1]Pediatrics; [2]Reproductive Biology; [3]Aerospace and Mechanical Engineering, Case Western Reserve University, Cleveland, OH.*
Background: We have shown that separation of amnion from choriodecidua occurs as an integral part of the process of fetal membrane (FM) rupture. Spontaneous amnion and choriodecidual separation is seen in FM after both SVD and elective CS deliveries. The etiology of spontaneous separation is unclear but biochemical degradation at the amnion-choriodecidua interface or physical shear forces resulting from labor may contribute. Correlating the degree of spontaneous FM separation with clinical characteristics may be informative.
Hypothesis: We hypothesize that gestation length, duration of labor contractions, and clinical infection are directly related to the extent of spontaneous separation of amnion from choriodecidua.
Methods: FM from consecutive deilveries were cut off the placental disk. Separated areas of FM were cut from the intact areas. Both were weighed and their weight ratios determined. Maternal medical, pregnancy and delivery data were collected.
Results: 141 FM had following associated characteristics: maternal age 26±6.7 yr, gravida 3.4±2.4, CS 15.6%, ROM 381±421 min, contractions 556±453 min, gestation 38±1.6 wks, African American 45%. 39% FM had less than 10 % separation, 30 % had more than 50 % and 7% had 100% separation. Infection, smoking, SROM, duration of ROM or contractions, delivery type and infant birth weight were not associated with increased separation of FM. However, meconium stained fluid (p=0.02), absence of epidural use (p=0.001), shorter duration from admission to delivery (p=0.04) and increasing gestation (p=0.007) were associated with increased FM separation. In FM from SVD (n=121), absence of epidural use (p=0.0009) or pitocin induction (p=0.03), shorter ROM duration (p=0.03) or duration admission to delivery (p=0.003) and increasing infant gestation (p=0.002) were associated with increased FM separation.
Conclusions: Mature pregnancy was associated with increased FM separation. Contrary to our hypothesis, duration of labor was not associated with spontaneous separation of FM. Therefore, we speculate that biochemical changes may be critical in initiating the FM separation process. Without biochemical initiation in a particular area of FM, stretch forces do not cause the separation. Support NIH 048476.

39

Induction of Apoptosis by Tumor Necrosis Factor (TNF-α) and Interferon Gamma (IFN-γ) on Cultured Human Chorion Epithelial Cells. Amy P Murtha,[1] Liping Feng,[1] Bryan Yonish,[1] Phyllis Leppert,[*1] David Schomberg.[1,2] *[1]Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA; [2]Cell Biology, Duke University, Durham, NC, USA.*
Introduction: PPROM is associated with 20% of all perinatal deaths. We have previously demonstrated, in both term and PPROM subjects, accelerated apoptosis in the chorion laeve of fetal membranes in the presence of chorioamnionitis suggesting that inflammation may contribute to the destruction of this important cell layer, either through apoptotic or necrotic cell death. Rupture at the choriodecidual level is an important early step in the fetal membrance rupture process and it has been suggested that production of inflammatory cytokines, such as TNF-α and IFN-γ are a fundamental link in the association between pre-term labour and intra-uterine infection. T-helper 1 (Th1) cell induced cytokine (TNF-α, IFN-γ) response has been proposed to mediate reproductive failure. However, the interaction between the cytokines TNF-α / IFN-γ and human fetal membrane cells has not been well studied. We hypothesize that the inflammatory mediators, TNF-α and IFN-γ, are responsible for fetal membrane chorion cell death and tissue remodeling, which ultimately puts individuals at risk for preterm premature rupture of membranes (PPROM).
Methods: Fetal membrane samples were collected from term elective repeat cesarean deliveries and chorion and decidua cells are processed as previously published. Cultured chorion and decidua cells were treated with TNF-α / IFN-γ. Cell viability was determined using a cell viability assay with viability estimates performed in quadruplicate. Apoptotic cell death was confirmed with Caspase 3 ELISA and PARP Western blot analyses. Data were analyzed using paired T tests with significance defined as $P<.05$.
Results: In chorion, treatment with TNF-α or IFN-γ resulted in significantly decreased viability and increased caspase 3 expression and Parp cleavage. The effect is significantly enhanced by combination of TNF-α and IFN-γ, suggesting a synergistic effect between these two mediators. Moreover, the chorion cells are preferentially affected since the observed effect was significantly less in the decidua cells.
Conclusions: These studies provide the direct evidence for a preferential cytotoxic effect of TNF-α / IFN-γ on isolated human chorion epithelial cells through apoptosis.

40

The Mechanisms of NF-kappa B Activation in Human Amnion with Labour. Sheri E Lim, Yun S Lee, TG Teoh, Phillip R Bennett.[*] *Imperial College Parturition Research Group, United Kingdom.*
Introduction
We have previously shown that labour is associated with increased Nuclear factor kappa B (NFκB) activity in amnion which plays an important role in the regulation of pro-labour genes such as COX-2 and Interleukin-8.
Labour is associated with increased cytokine synthesis which activates NFκB. Mechanisms leading to the activation of NFκB in amnion seen after labour may be different to those acting before labour. It is the mechanism of NFκB activation in amnion prior to labour that may be a therapeutic target, to prevent preterm labour.
Primary amnion cells cultivated from tissue collected prior to the onset of labour show varying degrees of NFκB activation which probably reflects 'closeness to labour'. The activation of NFκB requires its translocation into the nucleus as well as undergoing post-translational modifications such as phosphorylation. To study how amnion NFκB is activated, samples were classified into three groups- low, medium and high according to the level of total nuclear p65 or nuclear p65 phosphorylation at serine (ser) 536. The expression or activation of various proteins involved in NFκB signaling pathways were then correlated to the activation status of NFκB.
Methods and Results
We collected amnion from 12 women undergoing elective caesarean section and established primary cell cultures. Activation of NFκB was defined as (i) total nuclear p65 or (ii) nuclear phosphorylated p65 (pp65) at ser 536. Immunoblots were probed for nuclear p65, pp65 at ser 536, p52, Rel B, IKBb1 and 2, p50, phosphorylated p50 (pp50) and for cytosolic phosphorylated IKK 1 and 2, IKBalpha (IKBa), IKBepsilon (IKBe), pp65 at ser 536, p50 and pp50.
In the samples classified according to total nuclear p65 levels, there was a strong positive correlation with nuclear pp65 at ser 536 and nuclear Rel B and a weaker correlation with cytosolic pp50. When classified using pp65 at ser 536, there were strong correlations with nuclear p65 and cytosolic pp50, IKBa and IKBe expression.
Conclusions
Activation of NFκB in amnion cells prior to labour involves p65, p50 and possibly Rel B but does not involve p52. Activation of NFκB is not mediated by IKBa degradation or IkBb 1 or 2 synthesis and appears to be independent of IKK activity. The increased expression of IKBa and IKBe in cells with increased pp65 at ser 536 suggest that phosphorylation at ser 536 may be important for IKBa/e expression and that they act as reporters of NFκB activity rather than as inhibitory molecules.

41

Collagen Architecture-Mechanical Relations in the Fetal Membrane. Erinn M Joyce,[1] Michael S Sacks,[1] John J Moore.*[2] [1]*Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Departments of Pediatrics and Reproductive Biology, Case Western Reserve University, Cleveland, OH, USA.*

Objective: Fetal membrane (FM) premature failure accounts for one third of all premature human births and affects 3% of all pregnancies. In order to provide treatment and possible prevention of premature FM failure, we need first to understand FM structural and mechanical behavior its constituent layers near full term under normal physiological loading states. Once these properties are established, we can then better formulate how the tissue transitions to the ability to fail at full term.

Methods: We used small angle light scattering (SALS) to nondestructively quantify collagen fiber architecture of both the intact and separated FM layers. For mechanical evaluation we utilized planar biaxial mechanical testing to more fully simulate the FM physiologic loading state. Physiological loading tensions were approximated using the Law of Laplace and were applied to the intact and separated layers.

Results: In the stress free state, gross collagen fiber architecture of the FM and the separated layers were not homogenously aligned, but exhibited small regions of fiber alignment. The amnion layer displayed greater collagen fiber alignment than the intact FM and the choriondecidua layers. Under planar biaxial tension, specimens exhibited substantial mechanical anisotropy. As the tension level increased, the degree of anisotropy increased, which indicates that FM collagen fibers can undergo large rotations. We also observed that the initial nonlinear "toe" region of the stress-strain curves was small, and followed by a rapid transition into a highly linear region. These findings suggest that FM collagen fibers are only minimally crimped, and thus straighten rapidly, producing primarily linear stress-strain curves. Further investigation of the linear region suggested the FM collagen fibers are very straight at physiological loading ranges, which were well below known failure membrane tensions.

Conclusion: This study provided the first data on the planar biaxial mechanical characteristics of the FM. Our results suggest the FM collagen fibers become fully loaded and are straightened well below physiological loading levels. This result suggests little or no structural reserve in the FM, and may be an important aspect of its function and failure properties. This will also serve to guide future studies of the FM failure properties. Supported by NIH 48476.

42

Ultrasonographic Evaluation of Fetal Membranes Thickness. Caterina Bocchi, Filiberto Maria Severi,* Chiara Voltolini, Pasquale Florio,* Arianna Dell'Anna, Carmen De Falco, Felice Petraglia.* *Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy.*

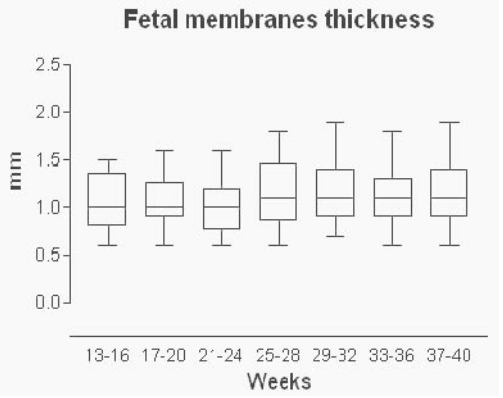
Objective. The thickness of fetal membranes (FMT) in pregnant women has been poorly studied by ultrasound and the distribution characteristics of sonographic measurements of fetal membranes (amnios, chorion) during normal gestation has not been yet quantified. The purpose of the study was to evaluate FMT measured by high resolution ultrasound throughout normal pregnancy.

Methods. In this prospective study a group of singleton pregnant women (n=245), with gestational age (GA) between 13-40 wks, was consecutively enrolled. Women whose pregnancy was complicated by any feto-maternal pathology were excluded. One ultrasound exam was performed to each patient to measure membranes thickness by using an high-resolution ultrasound equipment (myLAB50 Esaote SpA; Genova, Italy). The measurement of FMT was performed at about 3 cm from the umbilical cord insertion. After an high magnification of the image, the measurement was taken with the inner border of the horizontal line of each caliper placed on the line that defines the internal margin of chorion and amnion membranes.

GA was assessed by the LMP and confirmed by ultrasound. Data were expressed as mean ± standard deviation. The correlation between FMT and GA was evaluated by using Spearman test and differences among all gestational ages sub-groups was tested by using one-way ANOVA test.

Results. All women delivered at term (≥37 wks) normal fetuses. Maternal age was 37.2 ± 4.9 years. Mean GA at delivery was 39.2 ± 1.4 wks. Mean fetal weight was 3306 ± 453 g. On total population the mean FMT was 1.1 ± 0.3 mm (minimum 0.6; maximum 1.9). No statistically significant correlation was found between FMT and GA (p=0.11; r=0.10) (Fig. 1). Following the division into 7 groups in relation to GA (13-16; 17-20; 21-24; 25-28; 29-32; 33-36; 37-40 wks) the Kruskal-Wallis test showed a p value of 0.28.

Conclusions. By using the newest high resolution ultrasound equipments it is now possible to evaluate in vivo fetal amnio-chorionc membranes. The thickness of fetal membranes is gestational age independent.



43

Regulated Changes in Crosslinks between Versican and Hyaluronan May Facilitate Cervical Ripening. Ki Young Ryu, Mala Mahendroo.* *Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Hypothesis: Changes in the composition of the extracellular matrix control cervical remodeling during pregnancy and parturition. Associated with the changes in tensile properties of the cervix are changes in the collagen and glycosaminoglycan (GAG) components of the cervical connective tissue. The GAG, hyaluronan (HA) increases in the cervix during late pregnancy in numerous species. In other cell systems where HA plays a role in matrix structure and hydration, HA must interact with HA binding proteins in order to provide a stable structural matrix. Versican is a proteoglycan that forms crosslinks with HA. Enzymatic degradation of versican is carried out by members of the disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) family. In particular ADAMTS-1 has been shown to degrade the proteoglycans aggrecan and versican. Cleavage of versican by ADAMTS-1 severs the matrix crosslinking properties of versican. We propose that HA and versican crosslinks are required to disperse collagen fibrils during cervical ripening. In the current study, characterization of versican expression and degradation during pregnancy and parturition in the mouse will be determined.

Methods: Versican transcripts will be analyzed by real time PCR and 5'RACE. Cell specific expression will be determined by in situ hybridization. Protein will be detected by immunohistochemistry. Protein blots will be used to measure changes in ADAMTS1 expression.

Results: Versican mRNA is expressed throughout the second half of gestation and expression is increased shortly after birth on gestation day 19. Multiple isoforms for versican exist including the V0, V1, V2, V3 isoforms. PCR experiments using isoform specific primers have determined that the V1 isoform is the major isoform in the pregnant cervix. Immunohistochemical detection of versican localizes the protein to the cervical stromal matrix on gestation day 18. Our studies in the mouse cervix indicate that the mRNA and protein for ADAMTS-1 are increased in the cervix on gestation day 18, one day before onset of labor.

Conclusions: HA and versican crosslinks are required to disperse collagen fibrils during cervical remodeling. Upon degradation of versican by ADAMTS-1, there is a loss of HA: versican crosslinks which may be important in the postpartum remodeling of the cervical extracellular matrix to the nonpregnant state.

44

Lipid Peroxidation, Total Antioxidant Ability, and Antioxidant Vitamins in the Venous Plasma and Amniotic Fluid of Pregnant Women with Preterm Premature Rupture of Membranes. Yoon Ha Kim,*[1] Tae-bok Song,[1] Cheol H Kim,[1] Kwang S Lee,[1] Sung Y Yang,[2] Bong W Ahn.[2] [1]*Ob/Gyn, Chonnam National University Medical School, Gwangju, Republic of Korea;* [2]*Biochemistry, Chonnam National University Medical School, Gwangju, Republic of Korea.*

Objective: This study was performed to investigate the lipid peroxide levels, oxygen-radical absorbance capacity (ORAC) values, and antioxidant vitamin levels in the venous plasma and amniotic fluid of women with preterm premature rupture of membrane (PPROM) and to evaluate their roles of pathophysiology in PPROM.

Methods: Both samples of venous plasma and amniotic fluid were obtained from 20 normal pregnant women and 20 women with PPROM between 25 and 37 weeks gestation. Lipid peroxide levels were measured by thiobarbituric acid reaction. The ORAC values were measured by Cao's method and antioxidant levels were measured by high performance liquid chromatography.

Results: Lipid peroxide levels in the venous plasma and amniotic fluid of women with PPROM were significantly higher than that of normal pregancy (3.14±0.19 vs. 2.38±0.15 nmol/mg protein, $p<0.01$), (0.57±0.03 vs. 0.37±0.04 nmol/mg protein, $p<0.01$). The ORAC values in the venous plasma and amniotic fluid of women with PPROM were significantly lower than that of normal pregnancy (10,218.7±284.2 vs. 11,792.3±191.5 U/ml, $p<0.01$), (4,760.7±439.2 vs. 5,829.1±312.1 U/ml, $p<0.01$). The ORAC values/lipid peroxide levels in the venous plasma and amniotic fluid of women with PPROM were significantly lower than that of normal pregnancy (3,390.7±165.6 vs. 5,302.8±323.5, $p<0.01$), (9,166.2±1,219.2 vs. 22,858.3±3,850.8, $p<0.01$). Ascorbic acid levels in the venous plasma and amniotic fluid of women with PPROM were significantly lower than that of normal pregnancy (304.0±30.1 vs. 483.6±69.2 mol/ml, $p<0.05$), (1,279.8±267.6 vs. 3,136.2±216.5 nmol/ml, $p<0.01$).

Conclusion: This findings suggest that the imbalance of increased lipid peroxidation and decreased antioxidant activity in the maternal blood and amniotic fluid may be involved in the pathophysiology of PPROM. Low levels of ascorbic acid in the maternal blood and amniotic fluid appears to be an important determinant of PPROM.

45

Comparison of Human Uterine Cervical Electrical Impedance Measurements Derived Using Two Tetrapolar Probes of Different Sizes. Saurabh V Gandhi,[1] Dawn C Walker,[2] Brian H Brown,[2] Dilly O Anumba.*[1] [1]*Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom;* [2]*Biomedical Physics and Engineering, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

Background: Electrical impedance specroscopy (EIS) is finding increasing application in the study of the human cervix. Significant differences in cervical resistivity between normal and precancerous cervical epithelium have been reported using EIS probes of 5mm diameter. Studies which suggest that EIS may be used to assess pre-labour cervical ripening have employed probes of varied diameters (5, 8, and 9mm) on the assumption that deeper current penetration of stromal tissue may better detect the changes associated with ripening. No study has described the effect of varying probe size on cervical EIS values. We sought to a) compare uterine cervical resistivity obtained by a 5 mm and a 9mm probe, and b) employ finite element modelling to compare the fraction of injected electrical current that penetrates cervical stromal tissue.

Methods: Cervical impedance was measured in 12 subjects during early pregnancy using 2 different probes (5mm vs 9mm) on each subject, and was compared to a finite element model (FEM) of cervical histology and injected current.

Results: Mean cervical resistivity was significantly higher (5.4 vs. 2.8 Ohm.m, $p < 0.001$) with the smaller probe in the frequency range of 4-819 kHz. This difference was most marked at low (4kHz, mean ± SD resistivity 13.5 ± 3.9 vs 6.4 ± 3.9 Ohm.m respectively, $P < 0.01$), compared to high (819kHz, 2.26 ± 0.46 vs 2.0 ± 0.27 Ohm.m respectively, $P < 0.05$), frequencies. The cervical resistivity values obtained *in vivo* followed the pattern predicted by FEM. The latter confirmed that a higher fraction of injected current (%) penetrates cervical stroma for the 9 compared to the 5mm probe at all frequencies. Maximal stromal penetration was noted at 100kHz for both the 9 (90%) and 5mm (70%) probes.

Conclusions: The distance between the electrodes on EIS probes influences the resistivity values obtained, and the stromal contribution to those values. This may explain differences observed in previous studies. Consequently, the sensitivity of EIS for predicting labour inducibility and outcomes is likely to vary with probe size and design. This warrants further studies.

46

Catechol-o-methyltransferase Is Differentially Expressed in Fetal Membrane Tissues Obtained from Non-Laboring Versus Laboring Pregnant Women. Melissa J Wentz, Hassan Harirah, Ayman Al-Hendy.* *Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Introduction: During estrogen metabolism, Catechol-o-methyltransferase (COMT) converts the catechol estrogen, 2-hydroxyestrogen, to 2-methoxyestrogen. The 2-hydroxyestrogen can exhibit an anti-estrogenic effect while the 2-methoxyestrogen can exhibit an estrogenic effect depending upon the concentration of the estrogen metabolite and tissue type. Since COMT activity ultimately controls levels of these metabolites, it appears to be a key factor in regulating the cellular estrogenic milieu.

Objective: To investigate the expression of COMT in the amnion and chorion-decidua layers of fetal membranes obtained from laboring versus non-laboring pregnant women at term.

Study Design: Fetal membranes specimens were obtained from healthy term pregnant women who delivered at the University of Texas Medical Branch. Specimens were obtained from eight pregnant women after a normal spontaneous vaginal delivery and eight non-laboring women who underwent elective repeat cesarean section. The layers of fetal membranes were separated by peeling off the amnion layer from the underlying chorion-decidua layer. Total RNA was prepared from each specimen and used in RT-PCR assays. For each layer, the levels of COMT mRNA in samples from laboring pregnant women were compared to those from non-laboring pregnant women. Data was subjected to statistical analyses using Sigma Stat software. Protein lysates will be prepared from the tissue samples and used in Western blot analyses to confirm results obtained from the RT-PCR assays.

Results: Our preliminary results indicated that there was 3-fold higher expression of COMT mRNA in the amnion layer specimens from laboring women when compared to levels in specimens obtained during elective cesarean delivery (3.0 ± 0.7 versus 1.0 ± 0.4). This difference was statistically significant ($P < 0.05$). There was a trend for lower levels of COMT mRNA in chorion-decidua layer specimens from laboring women when compared to levels in specimens obtained during elective cesarean delivery (0.45 ± 0.12 versus 1.0 ± 0.3).

Conclusions: Our results suggest that the expression of COMT may be differentially regulated in the layers of fetal membranes during labor. This regulation may be necessary to block or enhance the action of estrogen in a tissue-specific manner during parturition.

47

A Comparison of Conventional ELISA(s) *vs* Microparticle Multiplex Immunoassays for the Determination of MMPs in Human Amniotic Fluid. Samuel S Edwin,[1] Katie Zeiter,[1] Adam J Pitt,[1] Rona Wang,[2] Lorri McLuckie,[2] Roberto Romero.*[2,3] [1]*Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA;* [2]*Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA;* [3]*Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, USA.*

OBJECTIVE: Increased concentrations of amniotic fluid (AF) matrix metalloproteinases (MMPs) have been observed in the context of premature rupture of membranes (PROM) and microbial invasion of the amniotic cavity (MIAC). MMPs belong to a family of potent matrix degrading enzymes and increased concentrations have been linked to premature birth and neonatal complications in human pregnancies. Concentrations of MMPs are generally determined using sensitive and specific microtiter plate based enzyme-linked immunosorbent assays (ELISAs). The present study was conducted to compare microparticle multiplex immunoassays capable of simultaneously quantifying 8 different MMPs in human AF with conventional MMP ELISAs.

METHODS: A mid-gestation human AF pool was prepared from remaining fluid following clinical testing procedures. Spike and recovery experiments were conducted in either assay buffer or varying concentrations of pooled mid-gestation AF using microparticle multiplex immunoassays and conventional plate ELISAs for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13. 360 AF samples without clinical identifiers were assayed for MMP-8 and MMP-9 using both measurement technologies.

RESULTS: Spike and recovery experiments yielded parallel curves for all of the MMPs tested using both the conventional plate ELISAs and microparticle multiplex immunoassay technologies. Microparticle multiplex immunoassays performed better on a larger dynamic range for all of the MMPs tested. Significant positive correlations were observed for AF MMP-8 (R=0.786; $p<0.0001$) and MMP-9 (R=0.947; $p<0.0001$) when the microparticle multiplex immunoassays were compared with conventional plate ELISAs.
CONCLUSIONS: Dynamic range of the assays, time, expense, and AF volume requirements clearly favor the multiplex immunoassay system. Simultaneous quantification of a panel of MMPs in AF utilizing microparticle multiplex immunoassays has potential implications for understanding the biology of MMPs in pregnancy complications.

48

PPAR-γ Activation Decreases Attachment of Endometrial Cells to Peritoneal Mesothelial Cells in an *In Vitro* Model of the Early Endometriotic Lesion. Shahryar K Kavoussi,[1] Anitha S Nair,[2] Craig A Witz,[2] Dan I Lebovic.*[1] [1]*Reproductive Endocrinology and Infertility/Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA;* [2]*Reproductive Endocrinology and Infertility/Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.*
Objective: Endometriosis affects approximately 10% of reproductive-aged women. Attachment of endometrial epithelial and stromal cells to peritoneal mesothelium appears to be the initial step in the genesis of the endometriotic lesion. We sought to determine if a PPAR-γ ligand, ciglitazone (CTZ), could reduce attachment of endometrial cells to peritoneal mesothelial cells.
Methods: The human endometrial epithelialoid cell line EM42 and peritoneal mesothelial cells (LP9) were used for co-culture experiments. EM42s were plated over confluent LP9s in 96-well plates. EM42s and/or LP9s were cultured with and without CTZ (10-40 μM) for 48 hours prior to co-culture. Four groups (n=8 replicates) consisted of: A) Control (no CTZ), B) CTZ treatment of EM42s, C) CTZ treatment of LP9s, and D) treatment of both EM42s and LP9s. EM42s were allowed to attach for one hour and an attachment assay was performed as previously reported. In addition, the rate of attachment of CTZ treated and untreated EM42 cells to hyaluronic acid (HA) coated 96-well plates was assessed.
Results(s): There was no difference in the rate of EM42 attachment when LP9 cells alone were treated with CTZ. When EM42s were treated with 40 μM CTZ, there was a 27% decrease in binding to LP9s ($P<0.05$). When both EM42s and LP9s were treated with 40 μM CTZ, there was a 37% decrease in EM42 attachment to LP9s ($P<0.001$). CTZ treatment of EM42s (40 μM) led to a 66% decreased rate of attachment to HA ($P=0.056$).
Conclusion(s): CTZ treatment of endometrial cells significantly decreased endometrial cell binding to peritoneal mesothelial cells and HA. Prevention of endometrial cell-mesothelial cell attachment could inhibit the genesis of endometriotic lesions. Further study of the role of thiazolidinediones in the treatment of endometriosis is warranted.

49

Disruption of Leptin Signaling Reduces the Microvascular Density of Endometriosis-Like Lesions in a Murine Model. AK Styer,[1] BT Sullivan,[1] RR Gonzalez,[2] M Puder,[3] D Arsenault,[3] Bo R Rueda.*[1] [1]*Vincent Ctr for Reproductive Biology Vincent OB/GYN Service, Massachusetts General Hospital, Boston, MA, USA;* [2]*Morehouse University School of Medicine, Atlanta, GA, USA;* [3]*Pediatric Surgery, Children's Hospital, Boston, MA, USA.*
Background: Elevated leptin levels have been observed in the peritoneal fluid of women with mild endometriosis, suggesting a role in the early stages of disease establishment. Furthermore treatment with leptin can increase vascular epithelial growth factor (VEGF) and its receptor (VEGF-R2) expression in endometrial cells.
Objective: Determine whether leptin is involved in the early angiogenic events that promote endometriosis, by investigating the effect of leptin signaling disruption on microvascular density (MVD) in a syngeneic mouse model of endometriosis.
Methods: Uterine tissue isolated from 6 wk old congenic C57BL6 mice primed with 10 U PMSG IP and euthanized 41 hr later, was placed in the peritoneal cavity of ovariectomized sister recipients with a SC E_2 implant. Treatment groups received pegylated leptin peptide receptor antagonist (LPRA) and controls scrambled nonfunctional pegylated leptin receptor peptide (scLPRA), IP (both at 66mM). Treatments included: daily IP administration starting 1 day prior to uterine tissue transfer and continuing for 7 days (continuous) or beginning 5 days after uterine tissue transfer and continuing for 48 hr (acute). Endometriosis-like lesions were removed from control and treated mice at day 7 post tissue transfer. MVD was determined on anti CD-31 stained frozen sections and counterstained with Hoerscht (1ug/ml). Utilizing fluorescent microscopy 3 random HPF (20X)/sample were selected, and the mean MVD (% of HPF) was calculated using IP Lab software (Scanalytics Inc.).
Results: MVD (%) was markedly decreased (>20 fold; $p<0.05$) in endometriosis-like samples from mice treated with LPRA (continuous; 0.225 +/-0.09) compared to those receiving scLPRA (4.55 +/-1.9). MVD was also decreased (4.5 fold; $p<0.05$) in the samples from LPRA group (acute treatment; 0.80 +/-0.29) compared to scLPRA (3.63 +/-1.2). A greater proportion of endometrial glands were observed in scLPRA versus LPRA in the continuous treatment arm.
Conclusions: *In vivo* disruption of leptin signaling significantly decreased MVD in endometriosis-like lesions. These findings strongly suggest a role for leptin involvement in small vessel recruitment during endometriosis lesion development.

50

Towards Gene Therapy of Endometriosis: Targeting Adenovirus to Human Endometriotic Cells Using Tissue Specific Promoters and Fiber Modified Viruses. Essameldine R Othman,[1] David T Curiel,[2] Ayman A Al-Hendy.*[1] [1]*OB-GYN, University of Texas Medical Branch, Galveston, TX, USA;* [2]*Gene Therapy Center, University of Alabama at Birmingham, Birmingham, AL, USA.*
Background:
Current medical treatment options for endometriosis are used only for short duration because of serious side effects. Gene therapy is a promising treatment alternative that has recently expanded in scope from malignant to benign diseases including leiomyoma and endometriosis. Adenovirus, a commonly used gene transfer vector, shows superior in vivo gene transfer but has a promiscuous tropism infecting many non-targeted tissues. In this study we examine the transduction of endometriosis cells by a panel of fiber modified and promoter modified adenoviruses that exhibit a liver-off profile to identify the specific modifications that sustain highest targeting to endometriosis cells.
Methods:
Human endometriosis and normal endometrial cells were grown in 12 well plates at a density of 50,000 cells per well. After 24 hour, cells were transfected with adenovirus serotype 5 (Ad-5), adenovirus under survivin promoter (Ad-Surv), adenovirus sigma (Ad-sigma) or adenovirus RGD (Ad-RGD) at MOI of 100 PFU/ cell. All adenoviruses used expressed luciferase as a reporter gene. 72 hours later, luciferase activity was measured in endometriotic and normal endometrial cells. The luciferase activity in cells transfected with each type of adenovirus was expressed as a percentage of the luciferase activity of the wild type virus (Ad-5)
Results:
In endometriosis cells, Ad-sigma showed luciferase tranactivation representing 133% of the of that induced by the wild type adenovirus whereas Ad-surv and Ad-RGD showed 11% and 22% of the luciferase activity of the wild type adenovirus respectively. In normal endometrial cells, Ad-sigma-mediated luciferase transactivation was 211% of that of the wild type virus whereas Ad-surv and Ad-RGD showed 11% and 42% of luciferase activity of the wild type virus respectively. Comparing the luciferase activity induced by Ad-sigma in endometriosis and endometrial cells, the luciferase activity of Ad-sigma in endometriosis cells was 4.5 fold higher than in endometrial cells.
Conclusions:
The fiber modified adenovirus sigma is more active in endometriosis and endometrial cells than the wild type adenovirus. Ad-sigma shows more selectivity to endometriosis cells than normal endometrial cells. Ad-sigma provides a promising vector for gene therapy of endometriosis.

51

The Role of ROCK II in the Development of Endometriosis. Cristina Rubiolo,[1] Silke Stadelmann,[1] Shima Djalali,[1] Daniela Piazzolla,[2] Rene Wenzl,[1] Andrea A Kolbus,[1] Johannes C Huber,[1] Walter Tschugguel.[*1] *[1]Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria; [2]Department of Microbiology and Immunobiology, Max F. Perutz Laboratories, Vienna, Austria.*

Introduction

ROCK II regulates cell contraction during migration in part by activating ezrin (E). We found that such an activation of E correlates with increased proliferation and migration of cells isolated from eutopic endometrium of patients (PEE) compared to those isolated from endometriosis-free controls (EFC) where E is not phosphorylated. These findings suggest an implication of ROCK II in the development of endometriosis.

Materials and Methods

Epithelial and stromal cell co-cultures were obtained from PEE (n = 8), corresponding ectopic lesions (EL; n = 8), and EFC (n = 8). ROCK II expression was partially and totally blocked in cells by Y27632 and by siRNA for subsequent WB and IF analyses against ROCK II, aromatase (Aro; both from St. Cruz) and phospho-ezrin (pE; Cell Signaling). The cells were as well analyzed for proliferation and migration.

Results

In cells derived from PEE the total inhibition of ROCK II expression associated with lack of pE, decreased Aro expression and impaired cell proliferation and migration. In contrast, cells from EL showed increased proliferation upon ROCK II inhibition. Moreover, while ROCK II was cytoplasmatically and perinuclearly distributed in cells from PEE, its localization was restricted to uropodia in both EFC and EL. The partial ROCK inhibition by Y27632 (10-6M) or the complete Aro inhibition by letrozole (10-7 M) caused the shift of ROCK II from perinuclear sites towards uropodia in cells from PEE, decreasing both cell migration and proliferation. pE was not detectable in cells of EFC, but was cytoplasmatically distributed in cells of PEE, and localized in the nuclei of cells from EL. Inhibition of ROCK II in cells from EL caused the shift of pE towards the cytoplasm.

Discussion

ROCK II affected the establishment of endometriosis partially by regulating E activation. Its distribution determines the rate of cell proliferation and migration in cells isolated from PEE and EL. While the perinuclear localization of ROCK II correlated with increased proliferation and migration of cells isolated from PEE compared to EFC, its partial inhibition caused its shift towards uropodia, thereby impairing cell proliferation and migration. This suggests that ROCK II effects are different according to its subcellular localization.

52

Interleukin-18 (IL-18) and IL-18 Binding Protein (IL-18BP) mRNA and Protein Expression in Eutopic and Ectopic Endometrium of Human Uterine Adenomyosis. Hong-Yuan Huang,[*1,2] Hsing-Tse Yu,[1] Tien-Hong Huang,[2] Chia-Woei Wang,[1] Chyong-Huey Lai,[1,2] Yung-Kuei Soong.[1,2] *[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Kwei-Shan, Tao-Yuan, Taiwan; [2]Obstetrics and Gynecology, Chang Gung University College of Medicine, Kwei-Shan, Tao-Yuan, Taiwan.*

Objective: Adenomyosis is a disease specifically characterized by deep invasion of the inner myometrium by endometrial glands and stroma thereby disrupting the endometrial-myometrial interface (EMI). IL-18 system is a major cytokine involved in human endometrium during menstrual cycle. IL-18 might perform a defensive role against maternal immune response in the uterine cavity. The purpose of this study is to investigate IL-18 and its antagonist, IL-18BP, expression in human eutopic and ectopic endometrium at the level of EMI in patients with uterine adenomyosis.

Methods: A total of 10 paired samples of human uterine eutopic; ectopic endometrium and corresponding normal myometrium were obtained from surgical specimens of women undergoing hysterectomy for uterine adenomyosis after informed consent and IRB approval. The uterine tissue samples used for this study were histologically shown to contain adenomyosis. Total extracted RNA was reverse transcribed and amplified by PCR using specific primers for GAPDH (94 bp), IL-18 (144 bp) and IL-18BP (188 bp). Real-time quantitative PCR was used to quantitative IL-18 and IL-18BP mRNA expression in paired human uterine samples. To determine the presence of IL-18 proteins, tissues were fixed and processed for immunohistochemical study. Data analysis was done with ANOVA and Pearson's correlation.

Results: IL-18 and IL-18BP mRNA were expressed in human eutopic, ectopic endometrium and normal myometrium. According to Real-time quantitative PCR with C_T value quantification, IL-18 was not significantly different in eutopic, ectopic endometrium and normal myometrium, but IL-18BP and the ratio of IL-18BP to IL-18 was significantly higher in normal myometrium in comparison to eutopic and ectopic endometrium ($p<0.05$). Immunoreactive IL-18 and IL-18BP at the protein levels was also present in paired tissue samples.

Conclusions: We have shown that IL-18 and IL-18BP expressed in the eutopic, ectopic endometrium and corresponding myometrium of uterine adenomyosis and a higher expression of IL-18BP in corresponding myometrium may implicate the involvement of the IL-18 system as a local immune regulator and modulating cytokine networks in pathogenic process of uterine adenomyosis.

53

Epigenetic Modification of Eutopic Endometrium in a Baboon Model of Endometriosis. Julie M Hastings,[1] Xiang Zhang,[2] Adrienne R Olson,[1] Kevin S Jackson,[1] Shuk-Mei Ho,[2] Asgerally T Fazleabas.[*1] *[1]Dept Ob/Gyn, Univ of IL, Chicago, Chicago, IL, USA; [2]Dept Environ Health, Univ Cincinnati Med Cen, Cincinnati, OH, USA.*

Introduction

Endometriosis is a gynecological condition associated with infertility. We have identified alterations in the endometrial expression of numerous genes and proteins following experimental induction of endometriosis in baboons. In particular, we have previously demonstrated that the reduced level of endometrial HOXA10 mRNA and protein in baboons with disease is associated with hypermethylation of the 5-UTR of the HOXA10 gene. We hypothesize that the development of endometriosis, and the resulting reduced fecundity, is associated with epigenetic modification of the endometrium.

Methods

Endometriosis was induced in 4 cycling baboons by inoculation of menstrual endometrium into the peritoneal cavity. Eutopic endometrium was consecutively harvested by laparotomy at day 10 Post Ovulation (window of receptivity) from baboons at 6 and 12 months post-inoculation. Control endometrium was similarly collected from disease-free animals. Aberrant DNA methylation patterns were identified by methylation sensitive restriction fragmentation (MSRF).

Results

MSRF revealed 14 hypo- and 2 hyper-methylated DNA fragments in baboons with endometriosis. BLAST analysis revealed sequence alignment of all 16 fragments to human and/or non-human primate DNA. Five of the 14 hypo- and 1 of the 2 hyper-methylated fragments displayed homology to known genes (Table 1).

Discussion

Hypomethylation and subsequent increased levels of genes involved in cell growth and differentiation may dysregulate differentiation of the secretory endometrium of baboons with disease. Hypermethylation and subsequent decreased levels of galectin 9 (LGALS9) may result in reduced immunotolerance and subsequent embryo rejection.

Conclusion

The development of endometriosis induces epigenetic modification of several genes within the eutopic endometrium; these alterations may contribute to the pathology of the disease and the pathophysiology of the resulting infertility.

(U54 HD40093 to ATF; NIH ES013071 to S-MH)

Table 1

Methylation Status	Gene Homology	Description/Funtion
Hypo	SHOX	Short Stature Homeobox
Hypo	WNT2B	Regulation of cell growth and differentiation
Hypo	TMEM97	Regulation of cell growth
Hypo	OR4D10	G-protein coupled receptor
Hypo	PLEKHG2	Regulation of Rho protein signal transduction
Hyper	LGALS9	Apoptosis of immune cells

54

The Role of PI3K Upstream of Ezrin Activation in the Development of Endometriosis. Cristina Rubiolo,[1] Silke Stadelmann,[1] Shima Djalali,[1] Daniela Piazzolla,[2] Rene Wenzl,[1] Andrea A Kolbus,[1] Johannes C Huber,[1] Walter Tschugguel.[*1] *[1]Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria; [2]Department of Microbiology and Immunobiology, Max F. Perutz Laboratories, Vienna, Austria.*

Introduction

Endometriosis correlates with increasing migration and proliferation of cells isolated from patients eutopic endometrium (PEE) as well as with enhanced transcription of aromatase (Aro) and subsequent activation of ezrin (E) through ROCK II. Here, we aim to investigate whether the action of Aro upstream of ROCK II requires the activation of PI3K.

Materials and Methods
Epithelial and stromal cell co-cultures were obtained from PEE (n = 10), corresponding ectopic lesions (EL; n = 10), and endometriosis-free controls (EFC; n = 10). PI3K function was blocked by wortmannin (400 nM) or LY294002 (0.02 mM), for subsequent WB, RT-PCR and IF analyses against ROCK II, Aro, ERs (all from St. Cruz) and phospho-E (pE; Cell Signaling). Cells were analyzed for proliferation and migration. The results were compared with those obtained upon ROCK II inhibition by Y27632 (0.1 mM) and siRNA and Aro inhibition by letrozol (100 nM).
Results
In cells derived from PEE the inhibition of PI3K associated with lack of pE and decreased Aro transcription, as upon letrozol treatment. PI3K inhibition associated with decreased translation of ER-alpha but not of ER-beta whereas it had not effects on their transcription rate; it resulted in severly impaired cell proliferation and migration, as upon ROCK II inhibition. Although this treatment showed the same effects on Aro in cells from EL, it did not affect protein levels of ER-alpha and ER-beta. In cells from EL, cell proliferation was only slightly increased and no differences between treated and non-treated cells were observed in cell migration. The inhibition of PI3K has no effect on cell proliferation and migration of cells isolated from EFC.
Discussion
PI3K affected the establishment of endometriosis by acting upstream of pE. Its effects are mostly related to the PEE where it strongly contributes to increase cell proliferation and migration, by controlling Aro transcription and ER-alpha translation. In contrast, in EL, PI3K only affects Aro transcription and slightly cell proliferation. Our data suggest that Aro and PI3K belong to a self-sustaining loop where the up-regulation of Aro increases the activation of PI3K and the inhibition of this kinase impairs Aro transcription.

55

Combination of CCR1 mRNA, CA125 and IL-6 Measurements in Peripheral Blood as a Diagnostic Test for Endometriosis. Admir Agic,[1] Christopher Altgassen,[1] Dietlinde Janson,[1] Monika M Wolfler,[2] Gulden Halis,[3] Klaus Diedrich,[1] Daniela Hornung.*[1] *[1]Obstetrics and Gynecology, University of Schleswig-Holstein, Campus Luebeck, Luebeck, Germany; [2]Obstetrics and Gynecology, University of Aachen, Aachen, Germany; [3]Endometriosezentrum Gendarmenmarkt, Praxisklinik für Fertilität, Berlin, Germany.*
Objective: Relying on our previous studies to find increased expression of CCR1 mRNA in peritoneal macrophages and peripheral blood leukocytes of women with endometriosis, we were prompted to investigate the possible use of CCR1 mRNA measurement in peripheral blood leukocytes, together with measurement of CA125 and IL-6 in serum, as a diagnostic test for endometriosis.
Methods: The expression of CCR1 mRNA in peripheral blood leukocytes was measured by quantitative real time PCR. CA125 and IL-6 levels in serum were determined by ELISA. 39 patients with endometriosis and 21 controls were enroled.
Results: The ratio of CCR1/HPRT (Hypoxanthine-Guanine Phosphoribosyl Transferase- housekeeping gene) mRNA in peripheral blood of patients with endometriosis was significantly elevated compared to women without endometriosis. The cut-off value for CCR1/HPRT ratio was 2.3, for CA125 40 U/ml and for IL-6 10pg/ml. The test was considered positiv for endometriosis if one of the markers was above the treshhold. This method showed a sensitivity of 94.9%, a specificity of 80.1%, a negative predictive value of 89.5% and a positive predictive value of 90.2% for predicting the presence of endometriosis.
Conclusions: Our results imply the potential use of CCR1 mRNA, CA125 and IL-6 measurements for the diagnosis or exclusion of endometriosis. Patients with an earlier diagnosis of this disease have a better treatment outcome and a reduced recurrence rate. Therefore, these measurements in the peripheral blood of patients with suspected endometriosis might give us a new perspective in diagnosing and treating this disease earlier and better.

56

Endometriosis Associated Progesterone (P) Resistance in the Oviduct: Evidence Based on the Localization of Oviductal Glycoprotein (OGP). Chaohua Wang, Patricia A Mavrogianis, Asgerally T Fazleabas.* *Dept Ob/Gyn, Univ of IL at Chicago, Chicago, IL, USA.*
Introduction: Endometriosis, a common cause of infertility, is associated with the development of an endometrial P resistance. Similarly to the endometrium, the oviductal function is critically governed by estrogen (E) and P. In the primate, E promotes hypertrophy and ciliogenesis of the oviductal epithelium with increased levels of OGP protein; in the luteal phase increasing P levels antagonize the actions of E. Furthermore, increased levels of oviductal ER and PR are present during the estrogenic follicular phase while P decreases both ER and PR during the luteal phase. We now hypothesize that the P resistance associated with endometriosis occurs in the oviduct.
Methods: Oviducts were harvested during the window of uterine receptivity (d 10 PO) from baboons 12-16 months following induction of experimental endometriosis (n=3) and from 2 animals with spontaneous disease. Oviducts were also obtained from control animals treated with the anti-P (ZK 137.299; n=3) and from animals during the follicular (n=8) and luteal (n=6) phases of the cycle. The tissue was processed for ICC localization using specific antibodies against ERα, PR and OGP. The epithelial cell height and the percentage of ciliated epithelial cells were also quantified on Gomori stained slides.
Results: OGP was significantly higher in animals with endometriosis compared to day 10 PO controls and were similar to that seen in late follicular phase and ZK treated animals. Baboons with spontaneous endometriosis showed a similar persistence of OGP (Table 1), which was correlated with the maintenance of ERα in animals with endometriosis. However epithelial cell height and the percentage of ciliation were not affected by endometriosis.
Summary: In the mid-luteal phase the presence of OGP in baboons with endometriosis indicates that P resistance occurs in the oviduct. These data suggest that the inhibition of P action may alter the oviductal environment and may be an additional factor that contributes to endometriosis-associated infertility. (U54 HD40093)

	Late Follicular	Mid Luteal	Exp Endometriosis	Spont Endometriosis	Mid Luteal + ZK	Mid Luteal + CG	Exp Endometriosis + CG
Cell Height (micron)	25.4 ±1.8	15.0 ±1.2	14.9±2.0	19.7±6.1	13.7 ±1.2	15.3 ±1.6	18.0±1.2
% Ciliation	55.5 ±3.9	3.6 ±1.6	3.7±1.0	18.3±0	5.9 ±0.8	7.1 ±1.2	6.7±0.4
OGP (H-SCORE)	232.4 ±8.5	38.9 ±11.9	212.7±30.4	217.5±25.5	189.4 ±45.4	38.3 ±15.0	207.3±63.3

57

Progestin Antagonist Therapy Suppresses Cell Proliferation and Growth in a Macaque Autograft Model for Endometriosis. Ov D Slayden,*[1] Rebecca L Caroll,[1] Ulrike Kaufmann-Reiche,[2] Ulrike Fuhrmann.[2] *[1]Division of Reproductive Sciences, Oregon National Primate Research Center, OHSU, Beaverton, OR, USA; [2]Female Health Care Research, Schering AG, Berlin, Germany.*
Objective: Endometriosis is a condition where endometrium-like tissues exist at ectopic sites outside the uterus. Autologous transplantation of macaque endometrium (Hum. Reprod. 11:150-164; 1996) produces endometrial grafts that can provide a nonhuman primate model for the disease. In primates, therapy with progesterone antagonists like ZK 230 211 (ZK; Schering AG) blocks estradiol (E)-stimulated cell proliferation in the eutopic endometrium (Hum, Reprod. 16:1562-74; 2001). Our goal in this study was to test the effect of ZK on E action in ectopic endometrial autografts. **Methods:** Eight adult ovariectomized rhesus macaques were treated with implants of E and progesterone (P) to induce 28-day, artificial menstrual cycles (Arch. Histol. Cytol. 67:393-409; 2004). On day 21 of the first cycle, explants of basalis zone endometrium were autografted to sites on the abdominal peritoneum. The grafts were allowed to establish and grow for three more cycles. At the end of fourth cycle the P implants were removed and the animals were treated with either E + vehicle (n=4), or E + 0.5 mg ZK/kg (s.c.; in Arachis oil; n=4) for 60 days. The grafts were collected on the last day of treatment, weighed, and prepared for histological and immunocytochemistry analysis. **Results:** Mean (±SE) serum E levels (91.4 ± 8.33 pg E/ml) were similar in both groups. ZK treatment significantly reduced graft wet weight compared to E_2-treated controls ($P<0.05$). Mean (±SE) graft wet weight in the E alone group and E + ZK group was 0.06 ± 0.1g and 0.01 ± 0.008 g, respectively. Histology revealed that this effect of ZK was associated with a reduction in the abundance and size of the ectopic endometrial glands. ZK significantly ($P<0.001$) blocked glandular cell proliferation detected by ICC for Ki-67 protein. Counts of Ki-67 positive epithelial cells revealed 22.6±6.1% in E alone and <1 ± 0% in E + ZK group. However, other markers of E action including estrogen receptor and progesterone receptor, detected by ICC, were unaffected by ZK treatment. **Conclusions:** ZK acted to block E-stimulated cell proliferation in ectopic endometrium but did not inhibit other endpoints of estrogen action and may provide a unique anti-endometriosis therapy.

58

The Elusive Diagnostic Test for Endometriosis – A Proteomic Approach. Monika M Wolfler,[1] Oliver N Richter,[1] Axel Wellmann,[2] Werner Rath,[1] Rene C Krieg.[2] (SPON: Walter Tschugguel). *[1]Obstetrics and Gynecology, Medical Faculty of the RWTH Aachen, Aachen, Germany; [2]Institute for Pathology, Medical Faculty of the RWTH Aachen, Aachen, Germany.*

Background: Endometriosis is one of the most common benign diseases among young women and yet underdiagnosed. Several screening markers have been introduced to decrease the interval between the onset of symptoms and the diagnosis of endometriosis. Changes on the proteomic level might precede the clinical manifestation of disease. Therefore, the analysis of these changes in patients´ serum using proteomic techniques like SELDI-TOF (surface enhanced laser desorption ionization time of flight) might contribute to the prediction of this disease in symptomatic patients.

Objective: We prospectively aimed to evaluate whether distinct patterns of serum proteins prior to laparoscopy in symptomatic women are of value to predict endometriosis.

Material and Methods: Serum of 91 consecutive patients attending this tertiary care centre for diagnosis and/or treatment of unexplained infertility, dysmenorrhoea, dyspareunia or chronic pelvic pain was collected. Prior to enrolment, estrogen dependent diseases other than endometriosis were clinically excluded by gynecologic exploration as well as transvaginal ultrasound examination. The serum samples were subjected to a standardized protocol. After mass spectrometric analysis according to SELDI-TOF standards by using a PBS IIc protein chip reader and Q10 as well as CM10 protein chip surfaces, analysis of data was performed by Ciphergen Proteinchip 3.1 and Bruker ClinProTools 2.0 Software.

Results: All samples were eligible for analysis. At laparoscopy, 50 out of 91 (54.9%) patients exhibited endometriosis and 41 (45.1%) were disease-free. Analyzing the serum samples, the software revealed a unique selection of mass peaks between 2 and 18kDa, which allowed for discrimination between patients suffering from endometriosis and controls with an overall recognition capability of 76.9% exhibiting a sensitivity of 66.7% and a specificity of 46.0%.

Conclusions: These preliminary findings provide direct evidence that screening for serum protein patterns using SELDI-TOF prior to laparoscopy could be of discriminative value in the prediction of disease. However, since endometriosis is not a systemic disease and serum protein expression is highly susceptible to interference, the proteomic analysis of other compounds might be of further interest.

59

Upregulation of Aromatase (*CYP19*) Expression by Cytokines and Androstenedione in Primary Explants and Cell Cultures of Endometrium: New Insight into the Pathogenesis of Endometriosis. Orhan Bukulmez,[1] Daniel B Hardy,[2] Bruce R Carr,[*1] Ruth A Word,[*1] Carole R Mendelson.[*2] *[1]Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA; [2]Biochemistry and Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA.*

Aromatase, the critical enzyme in estrogen biosynthesis from C_{19}-steroids, is upregulated in eutopical endometrium and is increased to even higher levels in peritoneal implants of women with endometriosis. Enhanced aromatase expression in endometriosis is likely mediated by an inflammatory cascade (i.e. increased NF-kB activation and cyclooxygenase-2 [COX-2] expression), which is associated with the pathogenesis of this disease. We postulated that *CYP19* gene expression may be enhanced by inflammatory cytokines and physiological levels of C_{19}-steroid substrate in the peritoneal fluid. To address this, we utilized human endometrium as an *in vitro* model. In the present study, we analyzed the effects of the inflammatory cytokine interleukin-1β (IL-1β) and the aromatase substrate androstenedione (A4) on COX-2 and aromatase mRNA levels in cell and explant cultures of endometrium obtained from hysterectomy specimens of pre-menopausal women and in the human endometrial-derived epithelial cell line (HES). Treatment of cultured endometrial explants, stromal and glandular cells with IL-1β (10 ng/ml) induced COX-2 and aromatase mRNA levels within 6h and 24h, respectively. To examine whether downstream products of the cyclooxygenase pathway mediated this effect, endometrial stromal cells were treated with cAMP and PGE_2, which also increased aromatase expression. Surprisingly, physiological concentrations of A4 (5-10 nM) markedly enhanced aromatase expression in endometrial explants, stromal and epithelial cell cultures, and in HES cells. A4 stimulation manifested within 24 h. Interestingly, A4 had no effect of COX-2 expression, suggesting that the stimulatory effects of IL-1β and A4 on aromatase are mediated by different mechanisms. Based on these findings, we suggest that inflammatory cytokines and androstenedione within peritoneal fluid may contribute to the marked increase in aromatase expression in endometriosis. Supported by NIH 5-R01-DK31206.

60

Increased C-Jun N-Terminal Kinase Activation in Endometriotic Endothelial Cells. Yesim H Uz,[1,2] William Murk,[1] Gulnur Kizilay,[2] Umit A Kayisli,[1] Aydin Arici.[*1] *[1]Ob, Gyn & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Hist & Emb, Trakya University School of Medicine, Edirne, Turkey.*

Background: JNK (c-Jun N-terminal kinase), a subfamily of mitogen-activated protein kinases (MAPK), is involved in many cellular processes ranging from cytokine expression to apoptosis, and is activated in response to cellular stress and inflammation. Endometriosis is an estrogen-dependent inflammatory disease. We have shown that human endometrial endothelial cells (HEEC) in ectopic implants have higher IL8 and MPC1 levels compared to those in normal tissues and their eutopic homologs. Our hypothesis is that there is increased JNK activation in endometriotic endothelial cells due to the inflammatory microenvironment of ectopic endometrium.

Objective: To determine phosphorylated- (P-) and total- (T-) JNK expression in endometrial and endometriotic endothelial cells. **Materials and Methods:** Normal (n=28), eutopic (n=8) and ectopic (n=12) endometriotic endometrial tissues were obtained from women undergoing surgery for benign gynecologic conditions or undergoing laparoscopy for infertility or pelvic pain. Paraffin sections were stained with T- and P-JNK antibodies and evaluated semi-quantitatively with HSCORE. Tissues were grouped according to menstrual cycle phase. Statistical analysis of the data was done using ANOVA and Student's t-test, with $p<0.05$ considered significant. **Results:** T-JNK immunoreactivity was both nuclear and cytoplasmic in the endothelial cells and did not show any change throughout the menstrual cycle. HEEC showed nuclear staining for P-JNK and expressed significantly higher P-JNK levels in the early proliferative (220±23) and late secretory (274±10) phases ($p<0.001$) compared to other phases (112±13). HEEC in ectopic implants showed significantly stronger P-JNK staining (250±6) compared to HEEC in normal endometrium (186±13, $p<0.05$). Moreover, HEEC in eutopic endometrial samples also revealed higher P-JNK immunoreactivity compared to HEEC in normal endometrium in the secretory phases ($p<0.01$). **Conclusions:** Increased expression of P-JNK activity in HEEC during early proliferative and late secretory phases correlates with elevated levels of cytokine expression in endometrium. Furthermore, these results support our hypothesis that increased phosphorylation of JNK in HEEC of ectopic implants may be involved in up-regulation of inflammatory cytokine expression and plays a role in the pathogenesis of endometriosis.

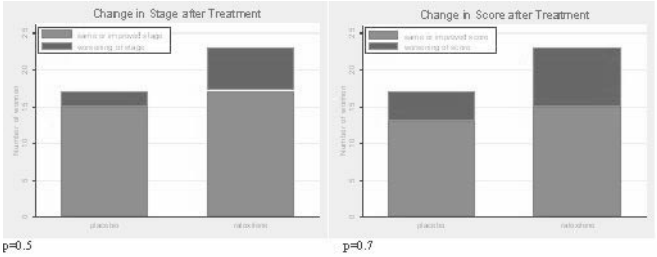
Support: None

61

Treatment with Raloxifene after Complete Surgical Excision of Endometriosis Does Not Lessen the Extent of Endometriosis at Second Surgery. Barbara J Stegmann,[1] Ninet Sinaii,[2] Lynette Neiman,[1] James Segars,[*1] Maria Merino,[3] Pamela Stratton.[*1] *[1]NICHD, National Institutes of Health, Bethesda, MD, USA; [2]Biostatistics & Clinical Epidemiology Service, National Institutes of Health, Bethesda, MD, USA; [3]NCI, National Institutes of Health, Bethesda, MD, USA.*

Objective: Raloxifene is an estrogen agonist-antagonist that does not stimulate the endometrium and, therefore, may inhibit endometriosis without lowering estrogen levels. We hypothesized that 6 months of raloxifene after surgical excision would inhibit regrowth of endometriosis. **Methods:** Women aged 18-45 with chronic pelvic pain and who had received no treatment for endometriosis in the previous 6 months underwent initial laparoscopy to confirm the presence of endometriosis and excision all lesions. They were then randomized to 6 months of raloxifene (180mg) or placebo daily. A second laparoscopy was performed after two years or earlier if pain returned. Return of pain was defined as two consecutive months of pain with severity equal to that experienced at study entry. Biopsy confirmation of endometriosis, rASRM stage and rASRM score were compared using logistic regression. Stage and score of disease were felt to show no change or to improve if the stage/score noted at the first surgery minus the stage/score of the second surgery was ≥ 0. **Results:** Of the 93 randomized women, 40 underwent a second laparoscopy. Eleven women (8 raloxifene and 3 placebo) had no biopsy evidence of endometriosis at second surgery ($p=0.2$). Overall, the raloxifene group experienced return of pain earlier and

had a significantly shorter interval between surgeries than the placebo group (522 days vs. 679 days, p=0.02). After adjusting for the interval between surgeries, there was no difference in the rASRM stage and rASRM score between the groups (p=0.5 and p=0.7, respectively) despite the earlier return of pain with raloxifene. **Conclusions:** Raloxifene after surgical excision shortened the time to return of pain but did not change the amount of endometriosis seen (as assessed by stage and rASRM score) between the women who received raloxifene vs. placebo.



p=0.5 p=0.7

62

Alterations in E-Cadherin (E-Cad) and Tissue Transglutaminase II (tTgase II) during the Window of Implation in a Baboon Model of Endometriosis. Kevin S Jackson, Patty A Mavorganis, Julie M Hastings, Asgerally T Fazleabas.* *Oby-Gyn, University of Illinois, Chicago, IL, USA.*

Introduction: Endometriosis is a condition associated with infertility. We have previously demonstrated dysregulation of several markers of uterine receptivity in a baboon model of endometriosis. Specifically, reduced levels of endometrial progesterone receptor and progesterone (P)-regulated genes, HOXA10 and calcitonin (CALC), were observed during the window of uterine receptivity in baboons with endometriosis, suggesting that this disease results in the development of an endometrial P resistance. In this study two CALC-modulated proteins, E-cad and tTgase II were evaluated in the eutopic endometrium of endometriotic animals throughout disease progression.

Materials and methods: Endometriosis was induced in normal cycling baboons (n=7) by intraperitoneal inoculation of menstrual endometrium. Eutopic endometrium was harvested consecutively from each animal at 1, 3, 6, and 15 months (m)of disease during the window of receptivity (d 8-11 post ovulation). Control eutopic endometrium was similarly harvested from disease free baboons (n=4).

Results: E-cad was immunolocalized in the glandular epithelium of both control and endometriotic eutopic endometria. However, E-cad immunostaining was markedly increased in endometria of baboons at 6 and 15m of disease. Strong stromal immunostaining for tTgase II was observed in disease free controls. A progressive loss of tTgase II immunostaining was observed throughout the time-course of disease.

Discussion: The maintained level of E-cad and reduced level of tTgase II correlates with the reduction of CALC previously demonstrated in eutopic endometria of baboons with endometriosis. E-Cad mediates calcium-dependent cell-cell adhesion. CALC reduces uterine E-cad in rodent epithelial cells and inhibits embryo implantation. Elevated levels of E-cad in baboons with endometriosis may increase epithelial integrity and render the endometrium unreceptive to implantation.Whilst the physiological role of tTgase II in pregnancy is not clearly understood, it is a direct target of CALC during the window of implantation in the rodent, and it is thought to mediate CALC-induced calcium-dependent protein crosslinking.

Conclusion: These data suggest that the reduced fecundity associated with endometriosis is mediated, in part, by a P resistance affecting not only genes directly regulated by P, but down-stream targets of P-regulated genes. (U54 HD40093).

63

Expression of L1 (CD171) in Endometriosis and Inhibition of Neurite Outgrowth by Anti-L1-Antibodies. Daniela Hornung,*[1] Admir Agic,[1] Songul Dogan,[1] Peter Altevogt,[2] Mina Fogel,[3] Dominique Finas,[1] Klaus Diedrich.[1] *[1]Obstetrics and Gynecology, University of Schleswig-Holstein, Campus Luebeck, Luebeck, Germany; [2]German Cancer Research Center, University of Heidelberg, Heidelberg, Germany; [3]Pathology, Kaplan Medical Center, Rehovot, Israel.*

Introduction

Endometriosis is frequently associated with serious pelvic pain such as dysmenorrhoea and dyspareunia. L1 (CD171) is known as a regulator of cell migration, proliferation, invasion and adhesion of several tumor cell types, but also as a stimulator of neurite outgrowth. We demonstrate the distribution and expression of L1 in patients with and without endometriosis. L1 might be an important mediator of the invasion, but could also be associated with increased nerve growth and pain in endometriosis.

Material and Methods

Endometrial and endometriosis biopsies were obtained from patients undergoing laparoscopy after providing written informed consent under a study protocol approved by the Luebeck Ethics committee. Immunohistochemistry and westernblot were performed on endometriotic lesions and endometrium using mAb L1-11A (subclone of UJ127.11). Stromal and epithelial cell cultures of endometriosis and endometrium were prepared for extraction of total RNA. Quantitative real-time PCR was performed with specific oligonucleotide primers for human L1. In a neuronal growth assay with chicken embryo ganglions, we tested conditioned media from endometrium epithelial cultures with and without L1 antibodies.

Results

Immunohistochemistry showed most intense L1 staining in atypical endometriosis, strong staining in normal endometriosis, weaker in endometrium from patients with endometriosis and the weakest L1 staining in endometrium from control patients. L1 staining was always prominent in epithelial cells and weak in the stromal compartment. Quantitative real-time PCR and westernblot confirmed these results. In chicken embryo ganglion assays, incubation with conditioned medium from endometriosis epithelial cells induced neurite outgrowth, which was inhibited by anti-L1-antibodies.

Conclusion

L1 could be involved in the adhesion and invasion of endometriosis tissue during the disease development and progress. Endometriosis associated pain might be mediated by increased nerve outgrowth. Future studies are needed to demonstrate, if L1 antibodies can be helpful for the treatment of endometriosis.

64

The Effects of Antioxidants Vitamin E and Vitamin C on Apoptosis-Related Gene Expressions in Endometrial Tissues. Mingqing Song,[1] Celia E Dominguez,*[2] Ana A Murphy.*[1] *[1]Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA, USA; [2]Department of Obstetrics and Gynecology, Emory University, Atlanta, GA, USA.*

Objective: *We propose to evaluate the effect of antioxidants such as vitamin* E and C on the expression of apoptosis-related genes in endometrial tissue.

Material and Methods: Immunohistochemical staining and RT-PCR analysis for the expressions of *Bcl-2, Bax, fas, caspace-3 and the TUNEL assay* in endometrial tissues.

Results: Staining for Bcl-2 in ectopic endometrium is significantly increased in women with endometriosis on placebo as compared to the normal group (3.13±1.03 *vs* 2.11±0.42, P<0.05). In eutopic endometrium of endometriosis subjects on Vit E, C, immunostaining for Bcl-2 was significantly decreased when compared to placebo group (2.17±0.44 *vs* 3.13±1.03, P<0.05). There were no significant differences in the staining between Vit E, C group and normal group.

Staining for *fas* and TUNEL in eutopic endometrium from placebo group was significantly decreased when compared to normal group (1.70±0.43 *vs* 2.96±0.80 and 1.69±0.66 vs 3.10±0.76; P<0.01). Staining in Vit E, C group showed a significant increase in immunostaining when compared to placebo group (2.85±0.37 vs 1.70±0.43 and 2.72±1.01 *vs* 1.69±0.66; P<0.01). There were no significant differences observed in immunostaining of eutopic endometrium between Vit E, C group and normal group.

The mRNA levels of Bcl 2-L-10, BAX, FAS, and Caspase-3 were analyzed in eutopic endometrial samples from endometriosis subjects either on vitamin E, C or placebo. A significant decrease in expression of Bcl-2-L-10 was noted in the Vit E and C group when compared to placebo (0.70±0.16 *vs* 1.04±0.17, P<0.01). A significant increase in *fas* mRNA was seen in the Vit E and C group when compared to placebo (0.74±0.07 *vs* 0.60±0.04, P<0.01). There were no statistically significant differences in BAX and Caspase-3 mRNA expression between vitamin E, C and placebo groups.

Conclusion: These data suggest that Bcl-2 and *fas* may be regulated by antioxidants. A pro-oxidant effect of these antioxidants is suggested by no significant difference between subjects with endometriosis on Vit E and C and normal women. This paradoxical effect of antioxidants, an increase in oxidative stress, can be seen with phenolic antioxidants such as Vit E and estradiol, as previously shown by Santanam et. al.

65

Serum Cytokines as Potential Bio-Markers for Non-Surgical Prediction of Endometriosis. Essameldine R Othman,[1] Daniela Hornung,[*2] Hosam T Salem,[3] Essam A Khalifa,[3] Tarek H Elmetwally,[4] Ayman A Al-Hendy.[*1] *[1]OB-GYN, University of Texas Medical Branch, Galveston, TX, USA; [2]OB-GYN, University of Schleswig-Holstein, Campus Luebeck, Luebeck, Germany; [3]OB-GYN, Assiut University, Assiut, Egypt; [4]Biochemistry Department, Assiut University, Assiut, Egypt.*

Background:
The gold standard for the diagnosis of endometriosis is diagnostic laparoscopy which is expensive, associated with possible surgical complications and has limitation in visualizing deep or retroperitoneal lesions. Overproduction of cytokines and growth factors has been implicated in the pathogenesis of endometriosis. The objective of this study is to test the ability of a group of serum cytokines to serve as biomarkers for the presence of endometriosis.

Materials and Methods:
Women undergoing laparoscopy for pelvic pain, infertility or tubal ligation were allocated to two groups according to post-surgical diagnosis: endometriosis patients and control women with no pelvic pathology. Blood samples were collected preoperatively and stored. Four cytokines were measured in the serum of endometriosis patients and controls: interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), vascular endothelial growth factor (VEGF) and tumor necrosis factor-alpha (TNF-α) using the Bio-Plex protein array system (Biorad). Non-parametric statistics were used to express the data

Results:
Two cytokines were significantly higher in serum of endometriosis patients than controls: IL-6 (4.41 versus 0.97 pgm/ml respectively, P<0.001) and MCP-1 (37.91 versus 22.13 pgm/ml respectively, P<0.001). There was no statistically significant difference between endometriosis patients and controls in the serum concentration of VEGF or TNF-α. None of the measured cytokines showed significant correlation with the cycle phase or stage of endometriosis. In a multivariate analysis, serum IL-6 provided the best discriminative ability between endometriosis patients and controls with a sensitivity of 71% and a specificity of 66% at a cutoff point of 1.9 pgm/ml. Adding MCP-1 to IL-6 did not increase the discriminative ability over that achieved by measuring serum IL-6 alone.

Conclusions:
Serum of endometriosis patients contains significantly higher levels of IL-6 and MCP-1 than control women. Serum IL-6 measurement discriminates between women with endometriosis and controls. IL-6 provides a promising serum marker for the non-surgical prediction of endometriosis.

66

Global Endometrial Gene Expression Analysis in Experimental and Spontaneous Endometriosis in the Baboon. Julie M Hastings,[1] Said Tabli,[2] Amy E Hamilton,[2] Ariane Germeyer,[2] Andrea G Braundmeier,[1] Kevin S Jackson,[1] Linda C Giudice,[*2] Asgerally T Fazleabas.[*1] *[1]Ob/Gyn, Univ of Illinois, Chicago, Chicago, IL, USA; [2]Ob/Gyn & Reprod Sci, Univ of California, San Francisco, San Francisco, CA, USA.*

Introduction
Endometriosis is one of the most common causes of infertility in women. To elucidate mechanisms whereby the presence of endometriotic lesions mediates reduced fecundity, eutopic endometria were analyzed by microarray during the window of implantation.

Methods
Endometriosis was induced in baboons (n=4) by intraperitoneal inoculation of autologous menstrual endometrium. Eutopic endometria were consecutively harvested at d9-11 post-ovulation at 1, 3, 6, and 15 months (m) after induction of disease and from 2 animals that spontaneously developed endometriosis. Control endometria were collected from animals with no disease (n=4). Total RNAs were individually hybridized to HG U133 Plus 2 Arrays (Affymetrix). Data was extracted using Affymetrix Gene-Chip Operating Software.

Results
Independent hierarchical clustering analysis with all samples revealed two major dendogram branches comprised of a) 1 and 3m and b) 6m, 15m, spontaneous endometriotic samples, together with controls. Unsupervised principal component analysis revealed self-clustering into two groups comprising of a) 1, 3 and 6m and b) 15m and spontaneous endometriotic samples, together with controls. A Heatmap of gene expression revealed dysregulation of many genes 1 and 3m after induction of disease, with lower levels of differential gene expression in 6m, 15m and spontaneous endometriotic samples. Pairwise comparisons at each stage of disease with control samples validated previous analyses showing dysregulation of several genes, including FOS and CYR61.

Discussion
Global analysis of gene expression in eutopic endometrium illustrates that the early stages of disease are associated with rapid and large scale gene dysregulation. In later stages, gene expression profiles are similar to those found in endometria harvested from animals with spontaneous disease.

Conclusion
These data demonstrate that the baboon model of endometriosis replicates spontaneous disease; furthermore, it provides a powerful means whereby the early events associated with the pathology of this disease and the pathophysiology of the resulting infertility can be elucidated. (U54 HD40093 to ATF; U54 HD31398 to LCG).

67

Endometrial Growth in Patients with Endometriosis during an IVF Cycle. Shelby Haugan, Ahmad Hammoud, C Matthew Peterson, Mark Gibson.[*] *Obstetrics and Gynecology, University of Utah Health Sciences Center, Salt Lake City, UT, USA.*

Background: Studies have shown that eutopic endometrium in women with endometriosis has altered cellular biochemistry and gene expression. Gross endometrial morphology is also different in patients with endometriosis. In this study, endometrial growth during in-vitro fertilization (IVF) was compared in cycles done for endometriosis and cycles done for male factor infertility.

Methods: We performed a historic cohort study. 73 women with laparoscopy confirmed endometriosis and 74 with male factor infertility were matched based on age. Data extrapolated included patient age, diagnosis, body mass index (BMI), estrogen level at the end of stimulation, and days of gonadotropin. Endometrial growth was measured by ultrasound endometrial thickness (ET) at the beginning of the IVF cycle and at the end of gonadotropin stimulation. Linear logistic regression was used to study the factors affecting ET, independent from confounders.

Results: The mean age of the patient population was 32.3 ± 0.36 years. Mean ET at the beginning of the cycle was 3.9 ± 0.12 mm and mean ET following stimulation was 11.4 ± 0.18 mm. The mean difference of ET between beginning and end of the cycle was 7.9 ± 2.25 mm for patients with endometriosis compared to 7.1 ± 2.45 mm in male factor infertility (p=0.032). Mean ET at the end of stimulation in patients with endometriosis was 11.8 ± 2.183 mm compared to 11.1 ± 2.25 mm in patients with male factor infertility (p=0.061). ET at the end of the cycle was divided into two groups based on the median, ≤11 and >11 mm. Patients with endometriosis were more likely to have an ET >11 mm than patients with male factor infertility, p=0.047 (Table 1).

After controlling for age, days of gonadotropin received, beginning ET, final estradiol level, and BMI, ET at the end of the cycle remained thicker in patients with endometriosis compared to male factor infertility (p=0.047). Notably, the estrogen level at the end of the stimulation did not correlate with ET at the end of the cycle.

Conclusion: The endometrial growth at the end of an IVF cycle was greater in patients with endometriosis compared to patients with male factor infertility. Patients with endometriosis had a higher frequency of thicker endometrium.

Table 1

	Endometriosis	Male factor infertility
>11 mm	41.1%	25.7%
≤11 mm	58.9%	74.3%

68

Expression and Localization of Nerve Growth Factor in the Endometriotic and Adenomyotic Lesions. Takashi Kajitani, Tetsuo Maruyama, Hironori Asada, Hiroshi Uchida, Hirotaka Masuda, Takashi Nagashima, Masanori Ono, Toru Arase, Kuniaki Ohta, Maki Kagami, Yasunori Yoshimura.[*] *Department of Obstetrics and Gynecology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan.*

Objective: Endometriosis and adenomyosis cause not only infertility but also acute- and chronic severe pelvic pain, compromising the quality of life in the patients. Molecular mechanism(s) underlying the endometriosis- and/or adenomyosis-related pain, however, remains elusive. Nerve growth factor (NGF) has been recently proposed as one of the key factors responsible for the onset and maintenance of chronic pain in a variety of tissues and diseases. In this study, to obtain a clue for the possible involvement of NGF in the generation of pain, we investigated the expression and localization of NGF in the lesions of ovarian endometriotic cysts, adenomyosis, and peritoneal endometriosis.

Methods: Eutopic endometrium, ovarian endometriotic cysts, and adenomyotic or peritoneal endometriotic lesions were obtained from consenting reproductive-aged patients with endomeriosis and/or adenomoysis who underwent laparoscopic or laparotomic surgery. Total RNA was extracted from these specimens and then subjected to real-time RT-PCR analysis. Also, the specimens were sectioned and stained immunohistochemically with NGF antibody. This study was approved by the Keio University Ethics Committee and all patients provided informed consent.
Results: Real-time RT-PCR revealed that NGF mRNA was significantly more abundant in the lesions of endometriosis and adenomyosis than the eutopic endometrium. Immunohistochemical analyses demonstrated that NGF was preferentially localized to the endometriotic glands of the ovarian endometriotic cysts and adenomyotic or peritoneal endometriotic lesions, while it was scarcely present in the eutopic endometrium.
Conclusions: Our results raise a possibility that NGF locally produced in the adenomyotic and endometriotic lesions may at least in part contribute to the generation of endometriosis- and adenomyosis-associated pelvic pain.

69

Trichostatin A, a Histone Deacetylase Inhibitor, Attenuates Invasiveness of Endometriotic Cells. Sun-Wei Guo,[1] Anna Starzinski-Powitz,[2] Yan Wu.[1] (SPON: Asgerally T Fazleabas). *[1]Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA; [2]Humangenetik fur Biologen, der Goethe Universitat, Frankfurt, Germany; [3]Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA.*
That endometriotic cells tend to be anti-apoptotic and pro-survival has been well recognized. The chronic inflammatory nature of endometriosis also has been documented. The current medical therapies all suppress proliferation of endometriotic and endometrial cells and appear to inhibit secretion of proinflammatory cytokines and chemokines by endometriotic cells. An insipid, and perhaps no less sinister, feature of endometriotic cells is its invasiveness, which has been reported a decade ago yet surprisingly little attention has been paid to contain this behavior through pharmacological means. In light of our findings that trichostatin A, a histone deacetylase inhibitor, suppresses proliferation, IL-1β-induced COX-2 expression, and constitutive or TNFα-stimulated NF-κB activation in endometrial and endometriotic cells, we sought to determine whether TSA can suppress the invasiveness in two endometriotic cell lines known to be invasive and E-cadherin negative. E-cadherin negativity is thought to be a marker for invasiveness in endometriotic cells. We found that TSA attenuates the invasiveness of two cell lines in the presence or absence of TNFα stimulation. In addition, TSA treatment reactivates E-cadherin expression in these cell lines. Since invasion may well be an important feature that holds the key for disease progression and recurrence, the complete eradication of endometriotic cells may require attenuation or complete suppression of invasiveness of endometriotic cells. Our results, along with our findings that TSA suppresses proliferation, IL-1β-induced COX-2 expression, and constitutive or TNFα-stimulated NF-κB activation in endometrial and endometriotic cells makes histone deacetylase inhibitors a promising class of compounds for novel and more effective medical treatment of endometriosis, especially given the mounting evidence that endometriosis appears to be an epigenetic disease.

70

Aberrant Nucleolin and Telomerase Expression May Affect Endometrial Function and Contribute to the Pathogenesis in Endometriosis. Dharani K Hapangama,[1] Jo Drury,[1] Carmen M Martin-Ruiz,[2] Siobhan M Quenby,[*1] Thomas Von Zglinicki.[2] *[1]School of Reproductive & Developmental Medicine, University of Liverpool, Liverpool Women's Hospital, Liverpool, United Kingdom; [2]Henry Wellcome Lab. for Biogerontology Research, University of Newcastle Newcastle General Hospital, Newcastle upon Tyne, United Kingdom.*
Aims/Objectives: We aimed to assess the importance of endometrial Nucleolin expression, telomerase and telomere length (TL) in endometriosis, and to immuno-localise nucleolin in the endometrium.
Materials and methods: In this prospective pilot study, endometrial biopsies were collected from 27 healthy, fertile, symptoms free women without endometriosis (Group 1, confirmed by laparoscopy) and 27 women with symptomatic, surgically diagnosed endometriosis (Group 2). 15 women in group 1 and 15 women in group 2 had biopsies taken during the cycle day 21+/-2 (implantation window). Further 12 women in group 1 and 12 women in group 2 were biopsied in the peri-menstrual period (day 26 +/-2). Endometria were dated according to recent modifications of Noyes criteria by two experienced pathologists[1]. Commercially available monoclonal anti-nucleolin antibody (NovaCatra, New Castle, UK), and rabbit poly clonal anti-telomerase antibody (Abcam, UK) were used in our immuno-histochemistry protocol. The mean TL was determined by qPCR.
Results, Summary/Conclusions
The endometria of fertile healthy women showed either weak or no telomerase or nucleolin immuno-reactivity throughout the luteal phase. Immuno-staining for both nucleolin and telomerase were significantly increased during the implantation window and the premenstrual endometria of women with endometriosis (nucleolin p =<0.001 for glands, epithelium and stroma, p=0.05 for endothelium; telomerase p =<0.0001 for all tissue compartments except perivascular region). The mean TL were significantly longer in endometrial cells of women with endometriosis during the implantation window (p<0.002). Aberrant endometrial nucleolin and telomerase expression may affect endometrial receptivity and contribute to pathogenesis of endometriosis.
To our knowledge this is the first report of immuno-localising nucleolin and hTERT in benign endometrium.
Reference
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71

Developmental Exposure to DES Permanently Programs TIMP-1 Gene Expression and Exacerbates Endometriosis in a Mouse Model. Susan C Nagel, Katie E Hurrelmeyer, Kathy L Sharpe-Timms,[*] Amy L Schroder, Jake M Redel, Ali L Ghormley, Hillary Myears. *The Department of Obstetrics, Gynecology and Women's Health, The University of Missouri-Columbia, Columbia, MO, USA.*
Objective: In the fetus, estrogens have permanent developmental effects, and our lab is investigating the potential for xenoestrogens to program endometriosis-related genes during development. Women exposed *in utero* to the synthetic estrogen diethylstilbestrol (DES) had an 80% increased incidence of endometriosis, an estrogen dependent disease characterized by the growth of endometrial tissue outside of the uterus. While women no longer take DES during pregnancy, human fetuses are exposed to many xenoestrogens, *e.g.* bisphenol A, during development. We hypothesize that developmental DES exposure permanently programs expression of endometriosis related genes and exacerbates endometriosis in adulthood.
Methods: Mice were developmentally exposed to a daily oral dose of 0.1 µg/kg DES or vehicle control. In adulthood endometriosis was surgically induced by auto-transplantation of uterine squares to the intestinal mesentery. After 4 weeks mice were euthanized and endometriotic lesions and eutopic endometrium were collected.
Results: In mice developmentally exposed to DES, the total endometriotic lesion mass was 60% larger than the endometriotic lesions from control mice. Also, expression of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) was programmed by developmental DES exposure. TIMP-1 gene expression was reduced 50% in endometriotic lesions of DES treated mice relative to controls.
Conclusion: TIMP-1 and matrix metalloproteinases (MMPs) have previously been shown to be aberrantly expressed in women with endometriosis. Our finding that developmental DES exposure decreased TIMP-1 expression in endometriotic lesions offers a potential mechanism for the increased lesion size as TIMP-1 inhibits MMPs that are necessary for the tissue remodeling associated with the establishment and growth of endometriotic lesions. This is an important and novel finding that developmental xenoestrogen exposure programs the fetus to exacerbate endometriosis in adulthood. Studies are underway to assess environmentally relevant xenoestrogens to which human fetuses are exposed. (Supported by NIH K01 DK60567and MU Research Council and Research Board grants to SCN)

72

Anomalous Oocyte Quality and Embryo Development in Rats with Surgically-Induced Endometriosis and Their Offspring Which Had No Surgical Intervention. Renita Woods-Marshall,[1,2] Julie A Weaver,[2,3] Peter Sutovsky,[2,3] Miriam Sutovsky,[3] Kathy L Sharpe-Timms.[*2,3] *[1]The Department of Veterinary Pathobiology; [2]The Department of Obstetrics and Gynecology and Women's Health; [3]The Division of Animal Sciences, The University of Missouri-Columbia, Columbia, MO, USA.*
INTRODUCTION: Classically, endometriosis has been associated with pain and infertility. Yet, whether endometriosis causes decreased fecundity remains one of the most controversial enigmas in reproductive medicine. Due to ethical restrictions, our ability to determine to mechanistic, causative answers is limited. Evidence suggests a genetic component exists for endometriosis yet conclusive verification is not present in the literature.

OBJECTIVE: Our objective was to examine whether compromised ovarian function contributes to anomalies in oocyte quality and embryo development and decreased fecundity in endometriosis. The architecture of the oocyte meiotic spindle and the proper alignment of chromosomes are both paramount to the success of early embryo development after fertilization, but have not been described in endometriosis patients. Consequently, we examined spindle and chromosome architecture in metaphase-II ova and pre-implantation embryos and determined whether oocyte anomalies are passed on to subsequent generations in an established rat model for endometriosis (Endo).
METHODS: Endo rats and surgical shams (Controls) were used (F0 generation, n=20 rats). Oocytes were obtained from the oviduct of estrus rats (day 0). Pre-embryos were obtained from pregnant rats on days 1, 3 or 5. Oocytes and pre-embryos were also collected from offspring of Endo and Control rats (F1 generation) that received no surgical interventions. Follicle and CL numbers were quantified morphometrically and the normalcy and quality of the oocytes and pre-embryos were evaluated by using the DNA stain DAPI combined with monoclonal anti-tubulin antibody E7 to evaluate chromatin integrity in interphase zygotes and spindle architecture and chromosome alignment in the mitotic pre-embryos and unfertilized ova with the use of epifluorescence microscopy combined with differential interference contrast (DIC). Statistical analysis was conducted by linear model ANOVA.
RESULTS: Endo rats had significantly fewer follicles and CL than Controls. Control day 0 oocytes had normal metaphase-II spindle formation whereas Endo oocytes had metaphase II ova with scattered chromosomes, cytoplasm fragmentation, spontaneous oocyte activation and formation of pseudo-pronuclei or karyomeres surrounded by a *de novo* formed nuclear envelope with nuclear pore complexes. Control day 1 zygotes had normal apposition of two pronuclei, where Endo zygotes had reduced fertilization rates or abnormal pronuclei with reduced pronuclear size, failed pronuclear apposition, misaligned chromosomes and nuclear fragmentation. Also in Endo rats, cytoplasmic fragmentation and delayed or arrested pre-embryo cleavage was observed between the first mitosis through blastocyst formation. At day 5, Control rats produced normal blastocysts with cytoplasmic microtubule networks, whereas only 6 to 8 cell pre-embryos were recovered from Endo rats on day 5 of gestation and cytoplasmic microtubules were not detected. Also, Endo rats had fewer implantation sites and more spontaneous pregnancy losses than Controls. Most intriguingly, F1 Endo offspring also had similar anomalies in fertilization, embryo development and reduced fecundity.
CONCLUSIONS: Endometriosis is associated with compromised ovarian function likely contributing to anomalies in oocyte quality and embryo development and decreased fecundity. These anomalies are also found in the F0 offspring born to Endo rats suggesting the presence of an epigenetic factor previously not identified with endometriosis.

73

WHI Replicated Using Observational Data from a Primary Care Practice Database, GPRD: Agreements and Disagreements. Kurt T Barnhart,[*1,3] Mark G Weiner,[2] Dawei Xie,[3] Richard L Tannen.[3] *[1]Dept of Ob/Gyn, Univ of PA, Philadelphia, PA, USA; [2]Dept of Medicine, Univ of PA, Philadelphia, PA, USA; [3]Dept of Clinical Epidemiology and Biostatistics, Univ of PA, Philadelphia, PA, USA.*
Introduction: Controversy exists concerning the validity of observational studies, highlighted by their discrepancy with the Women's Health Initiative regarding the cardioprotective effect of hormone replacement. This study was undertaken to rigorously address this issue with a comparison of a simulation of the WHI using observational data from a primary care practice electronic medical record with the results of the WHI.
Methods: A cohort from the United Kingdom General Practice Research Database (GPRD) was used to simulate the WHI by replicating, to the extent possible, all aspects of the RCT (subject selection criteria, study time frame, treatment and outcomes) except randomization. The simulated GPRD study examined 13,658 exposed subjects, treated with conjugated estrogen (0.625 mg daily) and norgestrel (150 µg on days 17-28), and 37,730 Unexposed subjects aged 55-79 and a sub study of 20,654 exposed subjects and 30,102 unexposed subjects age 50–55.
Results: In contrast to both previous observational studies and the WHI, myocardial infarction was neither increased nor decreased significantly in the GPRD Exposed Group [adjusted hazard ratio (0.95 (0.78-1.16)]. Similar to the WHI stroke [1.23 (0.99-1.52)], venous thromboembolic events [1.55 (1.37-1.75)], and breast cancer [1.67 (1.43-1.95)] were increased; and colorectal cancer was decreased [0.56 (0.35-0.87)]. Hip fracture [0.82 (.54-1.24)] was not significantly different from the RCT. Death was discrepant between the GPRD study and WHI with a lower risk of death in those exposed to HT in the analysis of the GPRD [0.75 (0.65-0.86)].
Results of the younger group of women were similar; HR 0.91 (0.69-1.20) for myocardial infarction, 1.52 (1.29-1.78) for breast cancer and 0.76 (0.63-0.91) for death.
Conclusion: A structured comparison of observational data from a large representative sample of the population exhibited outcomes that were largely concordant with the WHI but did not show a cardioprotective nor a cardioadverse effect of HT, and was associated with a lower risk of death. This study further generalizes, but is not in complete agreement the findings of the WHI. These findings demonstrate that a rigorous performed observational study that overcomes selection and other biases can provide valid results.

74

Estrogen Effects Post-Menopausal Women Differently Than Estrogen Plus Progestin Replacement Therapy: A Comparison of the WHI and a Large Primary Care Practice Database, GPRD. Kurt T Barnhart,[*1,3] Mark G Weiner,[2] Dawei Xie,[3] Richard L Tannen.[3] *[1]Department of Obstetrics & Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [2]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [3]Department of Medicine & Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.*
Introduction: In the Women's Health Initiative Randomized Controlled Trial (WHI RCT) treatment with estrogen-only differed from combined estrogen-progestin exhibiting less coronary artery disease, no increase in breast cancer and no reduction in colorectal cancer. Using the UK General Practice Research Database (GPRD) our previous simulation of the combined estrogen-progestin WHI RCT reasonably replicated this study. Therefore, the GPRD methodology was used to compare the estrogen-only and combined estrogen-progestin treatment.
Methods: The GPRD was used to simulate the estrogen-only WHI RCT of women with a hysterectomy to the extent feasible (subject selection criteria, study time frame, treatment and outcomes) except for randomization. The GPRD primary analysis examined 11,572 Unexposed and 6,890 Exposed women (ages 55-79) treated with Conjugated Equine Estrogen.
Results: In both WHI and GPRD studies, women with a hysterectomy exhibited increased cardiovascular risk factors and established cardiovascular disease at baseline and increased rates of cardiovascular outcomes. In the GPRD simulation of the estrogen-only WHI RCT the adjusted hazard ratios were 0.50 (0.38-0.67) for myocardial infarction, 1.13 (0.91-1.41) for breast cancer, 1.18 (0.72-1.92) for colorectal cancer and 0.68 (0.57-0.81) for death. Similar to comparison between the two WHI trials, these results differed significantly from the GPRD simulated combined estrogen-progestin study, by exhibiting less coronary artery disease, no increase in breast cancer and no reduction in colorectal cancer. Results were similar in the "Intention to Treat" and the "As-Treated" analyses.
Conclusions: The GPRD study confirms important differences in the response to estrogen-only as contrasted to combined estrogen-progestin treatment of postmenopausal women. Whether these differences result from different hormone regimens, differences in health status of women with a hysterectomy, or some combination thereof is unresolved.

75

Deficiency of an Ovarian Hormone, but Not Estrogen, Increases Myogenic Tone in Aged Female Rats. DJF Berezan,[1] Y Xu,[2] JR Falck,[3] Sandra T Davidge.[*2] *[1]Dept Physiology, University of Alberta, Edmonton, AB, Canada; [2]Depts OB/GYN & Physiology, University of Alberta, Edmonton, AB, Canada; [3]Dept Biochemistry, UT Southwestern Medical Center, Dallas, TX, USA.*
Background: Postmenopausal women are more likely to develop cardiovascular disease (CVD) than premenopausal women due to protective effects of estrogen on the vasculature. Aging is associated with arterial dysfunction including impaired responses to increased intraluminal pressure (myogenic tone). Moreover, ovariectomy in young adult rodents results in increased myogenic tone that is normalized with estrogen replacement. However, the combined effects of aging and ovarian status on myogenic tone have not been studied. 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor formed in vascular smooth muscle by cytochrome P450 4A (CYP4A) enzymes, mediates myogenic tone in mesenteric arteries from male rats; however, its role in females is unknown. We hypothesized that ovariectomy would result

in increased myogenic tone in aged female rats that could be normalized by estrogen replacement. We further hypothesized that 20-HETE would mediate these myogenic responses. **Methods**: Aged (16-17 months) female Sprague-Dawley rats were assigned to intact, ovariectomized (OVX) or ovariectomized and estrogen replaced (OVX-E) groups. After 4 weeks, resistance-sized mesenteric arteries were isolated and responses to intraluminal pressure (0-140mmHg) in the presence or absence of the CYP4A inhibitor DDMS (10μM) were assessed with a pressure myograph. **Results**: In agreement with our hypothesis, myogenic tone was increased in arteries from OVX rats relative to intact rats (% constriction: 18.0±5.6 vs. 1.8±0.9, p<0.001). Surprisingly, however, myogenic tone was not reduced by estrogen replacement in arteries from OVX-E rats (% constriction: 23.4±1.3, p<0.001). Furthermore, inhibition of CYP4A-dependent 20-HETE production had no effect on myogenic tone development in arteries from any group indicating that 20-HETE does not play a role in myogenic tone in arteries from female rats. **Conclusions**: Ovarian hormone deficiency increases myogenic tone in aged rats. In contrast to studies involving young rats, estrogen replacement did not reduce this reactivity. These data suggest that in aging females, deficiency of an ovarian hormone, but not estrogen, results in increased myogenic constriction. This may partially explain the increased risk of CVD in aging women even in the presence of estrogen replacement therapy.

76

The Mixed Meal Tolerance Test (MMTT)-Towards Superior Prediction of Glucose Intolerance in Newly Menopausal Women: An Ancillary to the Kronos Early Estrogen Prevention Study (KEEPS). Akas Jain,[1] Vlad Tomuta,[2] Lubna Pal,[1] Hussein Amin,[1] Ruth Freeman,[1] Daniel Stein,[2] Nanette Santoro.*[1] *[1]OB/Gyn, Division of REI, Albert Einstein College of Medicine, Bronx, NY, USA; [2]Medicine, Division of Endocrinology, Albert Einstein College of Medicine, Bronx, NY, USA.*

Objective: Identify a physiologic test incorporating 'real-life' nutritional content that correlates with accepted screening for diabetes mellitus in newly menopausal women.

Methods: Early menopausal women participating in the **KEEPS** trial were prospectively offered inclusion. At baseline, 12 healthy participants underwent a 75g oral glucose tolerance test (GTT) followed by MMTT. Fasting blood sugar (FBS) and HbA1c were obtained and blood glucose levels (BS) following MMTT and GTT were evaluated at 60 & 120 minutes for comparison. MMTT is a 600kcal shake containing 50% carbohydrates (75g glucose), 30% fat (vegetable oil) and 20% whey, followed by postprandial BS assessments among other metabolic measures (not reported in this abstract). Pair wise correlation analyses were performed to relate the screening tests to each other and to HbA1c levels. Multivariate linear regression modeling was used to determine independent predictors of outcomes. P-value <0.05 was considered statistically significant.

Results: Participants were 53.3±.5yrs old [SEM] and 1.2±0.2yrs past their final menses with BMI=26.2±1.0, waist/hip ratio (W/H)=.9±.03, HbA1c=5.4±.2% and FBS=89.3±3.4mg/dL. BS at 60 & 120mins following MMTT and GTT were respectively 131.7±4.5 & 122.9±.15.2 mg/dL, and 123.3±8.6 & 88.1±10.3 mg/dL. See table for correlation analysis between screening tests, FBS and HbA1c. The association between BS 120mins post-MMTT and HbA1c held on multivariate regression analyses adjusting for age, BMI, W/H and race (p=0.036). Neither FBS nor GTT parameters were predictive of HbA1c levels.

Conclusion: In healthy, recently menopausal women, MMTT but not the GTT predicts 'real-life' glucose tolerance as measured by HbA1c. These findings imply that the physiologic nature of the MMTT contributes to its sensitivity.

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Relationship of FBS and HbA1c with post-GTT and MMTT glucose

	Time (mins)	Correlation with FBS		Correlation with HbA1c	
		r-value	p-value	r-value	p-value
GTT	60	.46	.12	-.15	.62
	120	.69	.01*	.29	.34
MMTT	60	.61	.02*	.05	.87
	120	.71	.01*	.59	.04*

Pearson's correlation coefficient (r)=magnitude of association

77

Sonographic Changes of Uterine Artery Diameter and Uterine Vascularization during the Menopausal Transition and Early Postmenopause: Prospective, Four-Year Longitudinal Study. Anna Sokalska, Lil Valentin. (SPON: Antoni J Duleba). *Department of OB/GYN, Malmo University Hospital, Lund University, Malmo, Sweden.*

Objectives: To describe changes in uterine artery diameter and uterine vascularization during the period from 24 months before to 24 months after the menopause (MP).

Methods: 20 50 year-old women still menstruating underwent transvaginal gray scale/color and spectral Doppler ultrasound examination every 3 months until 12 months had elapsed since their last vaginal bleeding (MP); then every 6 months until 24 months after MP. Women were considered to be postmenopausal after 12 months of amenorrhea, provided that serum FSH was ≥ 17 IU/L (lowest reference value for postmenopausal women in our laboratory) in blood samples taken 3 and 6 months after the last vaginal bleeding. The examinations included measurements of uterine artery diameter, blood flow velocity and pulsatility index (PI) in both uterine arteries. The diameter of each uterine artery was measured three times from a longitudinal color Doppler image of the artery, the mean of the three measurements being used for analysis. The pattern of change over time for each variable was identified and noted for each woman. Statistical analysis was carried out using Wilcoxon Test.

Results: Three patterns of change over time were discerned: 1 -decrease, 2 –increase, 3 -no clear change. Pattern 1 was the most common for all variables except PI. Because observations for the right and left uterine artery were similar, only results for the right uterine artery are presented. Uterine artery diameter decreased during the 24 months preceding MP (P= 0.026) and continued to decrease after MP (P=0.044). Uterine artery peak systolic velocity and time average maximum velocity decreased over time with statistically significant changes occurring between MP and 24 months after MP (P=0.006 and P=0.008, respectively). Uterine artery PI did not change during study period (P=0.49). In all women FSH levels at 3 and 6 months after the last menses confirmed that the last bleeding was indeed the menopause; FSH levels ranging from 38 to 148 IU/L.

Conclusions: To our knowledge, this is the first prospective longitudinal study reporting perimenopausal changes of uterine blood flow. Decline of the uterine artery diameter occurred during the last two years prior to menopause and preceded decrease of flow velocity. We hypothesize that late premenopausal decrease of uterine artery diameter may be related to declining ovarian estrogen output.

78

Effect of Soy Protein with Isoflavones on Endogenous Hormones in Postmenopausal Women. Daniel R Christie,[1] Brian C Cooper,[2] Suzanne P Cliver,[1] John A Mahan,[1] Cynthia K Sites.*[1] *[1]Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL, USA; [2]Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*

OBJECTIVE: We sought to determine if a daily nutritional supplement of soy protein containing isoflavones would alter endogenous hormone levels in postmenopausal women.

METHODS: Eighteen postmenopausal women aged 55.6 ± 1.4 years with BMI of 30.5 ± 0.8 kg/m^2 (means ± SEM) were randomized to either a daily shake containing 20 g soy protein plus 160 mg isoflavones versus a daily isocaloric casein placebo shake for 3 months (Revival Soy, Kernersville, NC). Fifteen women completed the study and were analyzed at baseline and at 3 months (N=9 on soy, and N=6 on placebo). Fasting serum levels of LH, FSH, E2, and total testosterone (T) were measured by chemiluminescence (Access, Beckman Coulter). TSH and total thyroxin (T4) were measured by an automated chemiluminescence system (Centaur, Bayer). Sex hormone binding globulin (SHBG) was measured by equilibrium dialysis radioimmunoassay. Free T was calculated from the total T and SHBG. Serum isoflavones were measured by LC-multiple reaction ion monitoring-mass spectrometry. Means and SEMs at baseline and at three months, along with differences between means were compared between soy and placebo groups using the paired and unpaired Student's *t*-test for comparisons within and between groups, respectively. Significance was accepted with p<0.05.

RESULTS: There were no differences between groups at baseline. The increase in serum isoflavone levels was greater in the soy group vs. the placebo group (65-fold increase in genistein, p=0.01; 90-fold increase in daidzein, p=0.004; 42-fold in glycitein, p=0.005; 55-fold in dihydrodaidzein, p=0.002; and 46-fold in O-desmethylangiolensin, p=0.03). However, there were no differences between groups in TSH, T4, FSH, LH, E2, total and free T, or SHBG.

CONCLUSIONS: We conclude that a daily supplement of soy protein containing isoflavones markedly increases serum levels of several isoflavones without affecting the endogenous hormone levels of TSH, T4, FSH, LH, E2, total and free T, or SHBG in postmenopausal women. If confirmed in a larger population, our findings should offer reassurance that soy supplementation does not alter the risk of diseases linked to circulating levels of endogenous hormones.

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79

Resveratrol Induces G1-Phase Cell Cycle Arrest in Human Leiomyoma Cells. Huaijang Tang, John M Stribley, Gregory M Christman.* *Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA.*

An imbalance between proliferation and apoptosis is critical for leiomyoma formation. Estrogen receptor (ER) alpha is over expressed in leiomyomas. Resveratrol, a phytoalexin acts as a selective and potent antagonist of ER alpha and exhibits anti-neoplastic properties against a variety of tumor tissues.

Objective: To investigate the effect of resveratrol on cell proliferation, cell-cycle phase and apoptosis in human uterine leiomyoma cells.

Methods: Leiomyoma tissue was obtained from patients undergoing hysterectomy under an IRB approval protocol. Leiomyoma cells were isolated and cultured in DMEM containing 10% FBS. For proliferation assays, cells were plated at 15,000 cells in 24-well plates for 1 to 5 days, with resveratrol (0-150 uM), and cell viability was assessed using a sulfarhodamine B assay. To assess apoptosis and cell-cycle phase distribution, cells were plated at 100,000 cells in 6-well plates. After 24 hours, cells were treated with 0-150 uM resveratrol until harvested. Cells were evaluated for cell cycle phase using a flow cytometer. We examined protein expression of mediators involved in apoptosis or cell cycle regulation using western blot analysis.

Results: Leiomyoma cells underwent apoptosis after exposure to resveratrol. Proliferation assays indicated that resveratrol inhibits cell proliferation in a dose and time-dependent manner. Cell cycle phase analysis showed that there was a significant increase of the cell population in the G1 phase: 37.40 % (control) vs. 84.82 % (resveratrol) by day 3; and a significant decrease in the cell population in the S phase: 41.79 % (control) vs. 12.17 % (resveratrol) ($p<.05$). Western blot analysis showed that resveratrol induced protein expression of Bak, Bad, pRB, p21/Waf-1, p53, and p16; and reduced protein expression of Bcl-2, Bcl-xL Mcl-1, Cyclin E, Cyclin D1, Cdk2 and Cdk4.

Conclusion: Our results demonstrate that resveratrol induces apoptosis, and resveratrol-induced apoptosis is mediated by reduction of anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-2 and induction of pro-apoptotic proteins Bak and Bad in human leiomyoma cells. Our data also indicates that resveratrol arrests the cell cycle in the G1 phase and prevents progression from the G1 to the S phase. The growth-inhibitory effects of resveratrol appear to be mediated through up-regulation of pRB, p21/Waf-1, p53 and p16 and down-regulation of Cyclin E, Cdk2, Cyclin D1 and Cdk4. Supported by NIH RO1 HD46249.

80

Differences in Gene Expression and Functional Response of Retinoid Pathway Genes in Fibroid Compared to Myometrium. Marina Zaitseva, Beverley J Vollenhoven,* Peter AW Rogers.* *Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia.*

Microarray studies from our own and other laboratories have identified several members of the retinoid gene pathway that are differentially expressed between myometrium (M) and fibroid (F).

Objective: To investigate RNA expression, protein expression and localization, and in vitro regulation by retinoids of selected genes from the retinoid pathway in matched myometrium and fibroid samples.

Methods: Matched M and F were collected from hysterectomy specimens (n=25 total subjects), and snap frozen for RNA and protein isolation (n=12), fixed for immunohistochemistry (IHC, n=10), and used for isolatation of primary M and F smooth muscle cell (SMC) cultures (n=6). Gene expression was determined using real-time PCR, and protein expression by western blots and IHC. M and F SMC at passage 0 were treated with all-trans retinoic acid (ATRA) for 24 or 72 hours.

Results: Differential gene expression between F and M was confirmed for 2 binding proteins (CRBP1 and CRABP2), 3 enzymes (ADH1, ALDH1, RODH), and 1 responder gene (RARRES2) involved in the retinoid pathway. There were no differences in expression for the retinoid receptors (RAR-α, -β, -γ or RXR-α, -β, -γ). Differences in protein expression were confirmed by western blot for CRABP2 and ALDH1 but not for CRBP1. All 3 of these proteins were localized to cytoplasm of M, F and vascular SMC by IHC. In addition, ALDH1 exhibited intense cytoplasmic staining in fibroblast-type cells within connective tissue. Treatment of M and F SMC with ATRA increased CRABP2, CRBP1 and ALDH1 expression in a dose-and time-dependant manner, but did not affect ADH1 or RARRES2 expression. Fibroid SMC response to ATRA was similar to myometrial SMC for the 6 genes under investigation with the exception of ALDH1 at 72 hours, which was significantly reduced in F.

Conclusions: We have shown that a number of genes in the retinoid pathway show altered expression in fibroids compared to myometrium at both the mRNA and protein level. We have also demonstrated that there are limited but significant differences between the M and F SMC response to ATRA at a transcriptional level. Alterations in the retinoid pathway may potentially influence a large number of different downstream genes, however, it remains to be determined if aberrations in this gene pathway are a significant causative factor in the etiology of fibroids.

81

Testing of the Wound Healing Hypothesis for Leiomyomas: Evaluation of Versican Variants. John M Norian,[1,2] Minnie Malik,[1] Doina Joseph,[1] James H Segars,*[1,2] William H Catherino.[1,2] *[1]Obstetrics & Gynecology, Uniformed Services University, Bethesda, MD, USA; [2]RBMB, NICHD, NIH, Bethesda, MD, USA.*

Objectives: Leiomyomas exhibit a large degree of extracellular matrix (ECM) disorganization and aberrant extracellular signaling. Versican, a hyaluronan-chondroitin sulphate binding proteoglycan, forms an integral part of the ECM that organizes the uterine cells in linear arrays between collagen fibrils and also serves as a repository for cytokines and growth factors important for leiomyoma proliferation. Different levels of versican variant (V0, V1, V2, and V3) expression have been noted with tissues which have undergone repetitive wound healing. We have previously hypothesized that leiomyoma development is related to disorganized wound healing. We therefore suspected that versican variants are altered in leiomyomas.

Methods: After IRB approval, we obtained spontaneous uterine leiomyoma and matched myometrium from patients undergoing hysterectomy. Primary cell cultures were generated and immortalized using recombinant retrovirus containing the E6/E7 open reading frames of human papillomavirus type-16. Expression of versican variants was analyzed by end point RT-PCR, quantitative RT-PCR, western blot and cytoimmunofluoresecence in both tissue and immortalized cell cultures.

Results: All four variants (V0, V1, V2, and V3) were expressed in leiomyoma and myometrial tissue. Variant V0 mRNA was upregulated 4.9 fold (±0.66, $p<0.05$) in leiomyoma tissue and 8.31 fold (±2.11, $p<0.05$) in cell cultures compared with patient matched myometrium. V0 and V1 were expressed at higher levels compared with V2 and V3 as observed by end-point multiplex RT-PCR. Differential expression of versican was also shown by western blot analysis. Localization of the variants by cytoimmunofluorescence in cell culture indicated that V3 was predominately localized in the nucleus.

Conclusions: Versican variants were differentially upregulated in leiomyoma. V0 and V1 were expressed at higher levels in both leiomyoma and myometrium. It has been suggested that differing levels of proteoglycan composition and amount in leiomyomas compared with matched myometrium contribute to the disorganized ECM by filling in the wider spaced and irregularly shaped collagen fibrils. Our data suggest that larger versican variants differentially affect ECM composition and thus, participate in disorganized wound healing and leiomyogenesis.

82

Randomized, Placebo-Controlled, Double Blinded, Parallel Trial of the Novel Selective Progesterone Receptor Modulator, CDB-2914, Significantly Reduces Fibroid Volume after 3 Months. Eric Levens, Qingxiang Wei, Wendy Blocker, Alicia Armstrong, Clariss Potlog-Nahari, Lynnette Nieman. (SPON: James H Segars). *Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA.*

Objective: Symptomatic uterine fibroids account for over 200,000 hysterectomies in the US, as there is no available long-term medical therapy. We hypothesized that chronic administration of the selective progesterone receptor modulator CDB-2914 (CDB) would reduce fibroid size.

Design: Randomized, placebo-controlled, double blind, parallel study

Materials and Methods: Inclusion criteria included symptomatic fibroids as defined by ACOG, regular cycles and contraceptive use. Exclusion criteria included pregnancy, hemoglobin <10 g/dL, current hormone therapy, rapidly enlarging uterus and FSH >20 IU/mL. MR images were obtained to record

fibroid number, location and volume, before starting study drug and within 2 weeks of surgery. Women took CDB at a dose of 10 or 20 mg, or placebo (PL) for 3 cycles, or 90 days if they became anovulatory. The percent change in volume was compared in fibroids ≥2 cm in diameter. Wilcoxon rank sum test and t-test were used as needed.

Results: After IRB approval, 17 women (71% Black, 29% White) met inclusion criteria; all completed the study. The six who received PL had similar mean age and BMI to CDB group. There were 67 fibroids ≥ 2 cm with complete MR data. When evaluating the size of the largest fibroid within groups, PL had an average increase of 16% [CI:-15%, 46%] while CDB had a 22% reduction [CI:-29%, -15%]. When comparing CDB to PL, CDB had a significant percent reduction in fibroid volume (p=0.0003). A subgroup analysis of CDB by fibroid size of 2 – 4 cm, 4 – 6 cm and >6 cm showed a volume reduction of 24, 13 and 27%, respectively. All CDB patients became amenorrheic; one had complex endometrial hyperplasia without atypia at surgery.

Conclusions: Compared to PL, CDB-2914 at 10 or 20 mg daily significantly reduced the size of fibroids by 22% after 90 days, and induced amenorrhea. Apart from endometrial hyperplasia without atypia in one woman, there were no complications and the medication was well tolerated. Thus, there may be a role for CDB-2914 in the short-term treatment of uterine fibroids.

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83

Towards Gene Therapy of Uterine Fibroids: Fiber-Modified Adenovirus Show Higher Expression in Human Leiomyoma Cells Compared to Matched Normal Myometrial Cells. Memy H Hassan,[1] Salama A Salama,[1] David T Curial,[2] Ayman Al-Hendy.*[1] *[1]Obestetric and Gynecology, UTMB, Galveston, TX, USA; [2]University of Alabama, Birmingham, USA.*

Introduction: The development of novel therapeutic strategies is imperative for uterine leiomyoma. In this regard, adenoviral (Ad) gene therapy is a promising approach.

Aim: screening of several modified adenoviruses to identify the most efficient and selective virus towards human leiomyoma cells to be used as candidate for delivering therapeutic genes.

Methods: As experimental models we used primary cultures of human leiomyoma cells derived from tumors samples collected at time of hysterectomy as well as telomerase-immortalized human liomyoma cell line. We also used a telomerase-immortalized human myometrial cell line as a control. Wild type adenovirus serotype 5 (Ad5-Luc) as well as some modified viruses, Ad5 RGD-luc and Ad5 Sigma -luc (fiber-modified viruses) as well as Ad survivin-luc, that carry liciferase reporter under survivin promoter, were propagated on 293 cells and purified by double CsCL density centrifugation. Various cell types were transfected with 10 and 50 PFU/cell of these viruses using transfection medium for 4 h then media were replaced with complete normal media, incubation was continued for 48 h, and luciferase activities were determined using luciferase enzyme assay systems, according to the supplier's protocol. The luciferase activities were normalized against the amount of protein.

Results: Our data demonstrated that both Ad5 RGD and Ad5 Sigma had high expression levels of luciferase activities in both primary and immortalized human leiomyoma cells at 10 and 50 pfu/cell when compared to wild type Ad-Luc. Additionally, these modified viruses demonstrated selectivity towards leiomyoma cells compared to myometrium at 10 pfu/cell. Collectively, Ad5sigma-luc was the most selective agent towards human leiomyoma cells. Ad-survivin promoter exhibited minimal activity in all tested cell models.

Conclusion: Significant infectivity enhancement could be achieved in primary and immortalized human leiomyoma cells by utilizing the Ad5 vector in which the Ad5 knob is switched for that of reovirus(Ad5 Sigma) or short poly peptide is attached to H I loop (Ad5 RGD). These modified adenoviruses are more selective towards human leiomyoma cells than myometrium cells. These data establish the foundation for rational development of infectivity enhanced Adenovirus-based gene therapy for uterine lieomyoma.

84

Outcome of Laparotomic and Laparoscopic Myomectomy. A Randomized Prospective Study. Massimo Candiani, Stefano Izzo, Enrico Betto, Jennifer Riparini, Nicola Berlanda. (SPON: Anna Maria Marconi). *Obstetric & Gaenecology, S. Paolo Hospital, Milan, Italy.*

Objective: To compare laparoscopic and laparotomic myomectomy, focusing on short and long term intra and post-operative parameters.

Methods: A prospective, randomized, controlled analysis of 60 consecutive patients with a uterine volume ≤ 300 cc and number of myomas ≤ 5 submitted to laparoscopic (1) and laparotomic (2) myomectomy with 12 months of follow up.

Results: Patients' mean age, body mass index, number of myomata and maximum diameter were comparable in the two groups. Operative time was camparable in the two groups (79 ±43mins in (1) and 78 ±19mins in (2)). There was a statistically significant difference for blood loss (98 ±87cc in (1) vs. 189 ±134cc in (2)p=0.003). For the post operatory hospital stay (2.3 days (1) and 3.6 days (2)p=0.001).

1 month follow up showed a statistically significant difference for pelvic pain and urinary disorders (53.3% in (1) Vs. 26.7% in (2)p=0.04), further painful symptoms (30% in (1) Vs. 6.7% in (2)p=0.007), mean days off of work (20 ±9.7days in (1) Vs. 25 ±15 in (2)p=0.03). 6 and 12 months follow up revealed comparable data for clinical evaluation and a pregnancy rate of 58.3 and 44.4 % in (1) and (2) respectively. The US evaluation revealed a persistence of 4.7% (1) and a 0% in (2) after single myomectomy; 22.4% in (1) and 21.4% in (2) after multiple myomectomy. After 12 months, there was 13% of relapses in (1) and 10% in (2).

Discussion: In uteri with a volumen <300 cc and myomatas number <5, laparoscopic myomectomy is better than laparotomic approach. In (1), lower blood loss, shorter hospitalization and convalescence and reduced post-operative adhesions risk were noticed. Persistence and relapse rate is more influenced by myomatas' number (single or multiple myomectomy) than surgical technique.

85

Estrogen Receptor Alpha (ERα) Phospho-Serine-118 Is Highly Expressed in Human Uterine Leiomyoma Compared to Myometrial Tissue. Tonia L Hermon,[1,3] Alicia B Moore,[1] Grace E Kissling,[2] Frank J Castora,[3] Silvina Bocca,*[4] Darlene Dixon.[1] *[1]Laboratory of Experimental Pathology, Comparative Pathobiology Group; [2]Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC; [3]Department of Physiological Sciences; [4]Jones Institute of Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA.*

OBJECTIVE: Uterine leiomyomas (fibroids) are the most common tumors found in the reproductive tract of premenopausal women and are the leading cause of hysterectomy in the United States. It has been suggested that their growth may be mediated by the interaction of hormone and growth factor pathways and that phosphorylation of the estrogen receptor alpha (ERα) at serine 118 may be important in this interaction.

METHODS: Immunohistochemical, western blot and immunofluorescence procedures were used to determine the expression of phosphorylated ERα Serine 118 (ERα-phospho-Ser118), phosphorylated p44/42 Map Kinase (phospho-p44/42) and proliferating cell nuclear antigen (PCNA) in human leiomyoma and patient-matched myometrial tissues (n=10) during the proliferative and secretory phases of the menstrual cycle.

RESULTS: We found that fibroids, overall, expressed more ERα-phospho-Ser118 ($p \leq 0.01$) and PCNA ($p \leq 0.05$) than myometrial tissue, and tumors from the proliferative phase had significantly increased expression of ERα-phospho-Ser118 ($p \leq 0.05$) than those from the secretory phase. Immunoexpression of both phospho-p44/42 ($p \leq 0.05$) and ERα-phospho-Ser118 ($p \leq 0.05$) was increased in the nuclei of tumor cells from the proliferative phase of the cycle compared to myometrial cells. There was also increased colocalization of phospho-p44/42 and ERα-phospho-Ser118 in the nuclei of tumor versus normal cells from the proliferative phase of the menstrual cycle.

CONCLUSIONS: These data suggest that phosphorylation of ERα at serine 118 may be important in leiomyoma growth and that this phosphorylation may be regulated by estrogen and progesterone. These data also imply that phospho-p44/42 may phosphorylate ERα at Ser118 due to the increased coexpression of both proteins in the nuclei of tumors versus myometrial cells. Understanding the interaction between ERα and growth factor pathways and how they regulate leiomyoma growth will help develop noninvasive therapeutic strategies for treatment of these tumors.

86

Leiomyomas May Impact Preterm Labor by the Expression of Fetal Fibronectin. Minnie Malik,[1] John M Norian,[1,2] Joy Britten,[1] James H Segars,[*1,2] William H Catherino.[1,2] *[1]Obstetrics & Gynecology, Uniformed Services University, Bethesda, MD, USA; [2]RBMB, NICHD, NIH, Bethesda, MD, USA.*

Objective: Leiomyomas are a highly prevalent disease associated with preterm labor. These tumors are made up of excessive and disorganized extracellular matrix (ECM). Fibronectin, a high molecular weight ECM glycoprotein, is involved in tissue repair, a process that is aberrant in leiomyomas. Fetal fibronectin (fFN), as a marker for inflammation involving ECM deposition, correlates with preterm labor risk. Multiple adult tumors can also express fFN. We hypothesized that fFN expression is elevated in human uterine leiomyomas.

Methods: After IRB approval, we obtained spontaneous uterine leiomyoma and matched myometrium from five patients undergoing hysterectomy. Primary cell cultures were generated and immortalized using recombinant retrovirus containing the E6/E7 open reading frames of human papillomavirus-16. Expression of fFN was analyzed by end point RT-PCR, quantitative RT-PCR, western blot, and cytoimmunoflouresence in myometrial and leiomyoma tissues and immortalized cell cultures.

Results: In surgical leiomyoma specimens, major fibronectin variant-1 was elevated 2-fold. In immortalized leiomyoma cell lines, we found similar over-expression (2.14 ±0.13 fold). By cytoimmunofluorescence, we found over-expression of fibronectin in leiomyoma cell lines compared with myometrium. Since fFN is more commonly associated with inflammation and early ECM deposition, we evaluated this variant by multiplex RT-PCR and found that fFN mRNA was up-regulated in pathological specimens of leiomyomas compared with matched myometrium irrespective of patient demographics. This finding was also confirmed in culture. Using western blot and cytoimmunofluorescence, fFN demonstrated enhanced expression compared with myometrium.

Conclusions: The expression of both fibronectin variant-1 and fFN were elevated in leiomyoma tissue and immortalized cell lines compared with myometrium. Increased expression of fFN is known to influence ECM signaling, inducing production of activated forms of matrix metalloproteinases, which may play a central role in fibroid formation. As both increased levels of cervical fFN and the presence of fibroids are associated with preterm labor, there is a potential molecular link between leiomyoma presence and preterm labor via fetal fibronectin expression.

87

Memy I: A Novel Animal Model for Uterine Leiomyoma Using Adenovirus-Enhanced Human Fibroid Explants in SCID Mice. Memy H Hassan, Salama A Salama, Ayman Al-Hendy.[*] *Obestetric and Gynecology, UTMB, Galveston, TX, USA.*

Background: Human uterine leiomyoma is the leading cause of hysterectomies in USA and it is underlying causes are poorly understood, in part due to lacking of a good animal model.The only curently available model is the Eker rat in which both leiomyosarcomas as well as leiomyomas are observed. The establishment of human leiomyoma in mouse models has not been successful until now.

Aim: Development of a reliable in vivo model of human leiomyoma using SCID mice implanted with human leiomyoma explants.

Method: Two month-old female SCID mice were supplemented sc with estrogen pellets. Fibroid tumor tissues were collected from patients at time of hystectromy, and confirmed by the surgeon and a pathologist. Fibroid tissues were cut into tiny wedge-shaped pieces. Wedges were randomly divided into five groups based on specific adenovirus transfection: group *I* transfected with Ad-LacZ (positive control for virus transfection), group *II:* Ad-VEGF, group *III*: Ad-COX2, group *IV* transfected with both Ad-VEGF & Ad-COX2, and group *V* treated with the transfection medium alone (negative control). Two wedges of tissue were dipped in Matrigel and inserted SC in the right flank of each animal. Mice were closely monitored and the developed lesion was measured biweekly. Random samples were collected at 14, 21 and the experiment completed at 30 days. xenograft tissues were harvested and examined regarding: gross evidence of vascularization, tissue morphology, proliferation, apoptosis, as well as expression of estrogen receptor(ER), and smooth muscle (SM) actin.

Results: Our data demonstrated that tissue wedges transfected with both Ad-COX2 and Ad-VEGF(group *IV)*, continued to grow throughout the duration of the experiment and were well vascularized. Immunohistochemical analysis revealed that the number of proliferating and apoptotic cells were similar between matched original patient tissue and xenografts recovered after one month in group *IV* . Additionally H&E staining revealed the characteristic pearl appearance of human leiomyoma in the SC grown lesions. ER and SM actin evaluation are in progress in our lab.

Conclusion: Human uterine leiomyoma xenografts are established in SCID mice for one month after pre-implantation transfection with Ad-COX2 and Ad-VEGF. The xenografts were well vascularized and maintained fibroid tissue architecture and the expression of key markers. This model represents a novel tool for *in vivo* study of human uterine fibroids.

88

Towards Fibroids Gene Therapy: Adenovirus Mediated Delivery of Dominant Negative Estrogen Receptor Shrinks Uterine Leiomyoma Tumors in Eker Rats. Memy H Hassan, Salama A Salama, Dong Zhang, Ayman Al-Hendy.[*] *Obestetric and Gynecology, UTMB, Galveston, TX, USA.*

Background: Uterine leiomyoma are benign smooth muscle tumors that originate from myometrium. Leiomyoma are sex steroid hormone-dependent neoplasms Like many other estrogen-responsive tumors, the effects of estrogens in leiomyomas cells are mediated by the estrogen receptor (ER), of which both subtypes, ERα and ERβ, were found in fibroids. Dominant negative ERs (DN-ER) are ER mutants that are unable to activate transcription and have the additional property of being able to suppress the transcriptional activity of the wild-type ER when they are co expressed in the same cells**.**

Aim: Assessment of the efficacy of in vivo gene therapy of uterine leiomyoma in the immune-competent Eker rat model using adenovirus mediated delivery of DN-ER (Ad-DN-ER).

Method: Ten Female Eker rats 11-14 months old with MRI-confirmed uterine leiomyoma lesions were randomized to a single treatment with direct intratumor injection of Ad-DN-ER versus Ad-Lac Z as negative control. The tumor volume was determined by serial MRI scanning and confirmed with caliper measurement. Animals were observed daily for any post-treatment complications. Sample rats were selected randomly and killed at the following time points 0, 8 and 15 day and experiments completed at 30 days. Tissue samples were collected from tumors for further evaluation as well as samples from other organs eg. uterus, ovary,spleen, etc. to assess the safety of the adenovirus treatment.

Results: Ad-DN-ER treatment produced dramatic shrinkage of the total uterine fibroid volume by -45%, -80% and -78% of pretreatment volume at days 8, 15and 30 respectively while the tumor size in negative control animals receiving Ad-LacZ continued to grow by +26%,, +66%, +102% at same time points. All animals well tolerated the virus inoculation and survived the experiment without any apparent sign of toxicity. The PCR detection assay of E4 region of the adenovirus revealed that the adenovirus transfection is localized to the fibroid tissues and did not disseminated to other organs

Conclusion: Adenovirus-mediated deliveries of dominant negative gene by direct intra-tumor inoculation shrink uterine leiomyoma lesions in Eker rats. The delivered virus particles do not disseminate to adjacent or distant organs. These studies provide essential preclinical data for the development of gene therapy as an alternative non-surgical treatment option for uterine leiomyoma.

89

Aromatase Expression in Uterine Fibroid Cells Is Regulated by Promoter Elements That Bind CAATT/Enhancer Binding Proteins (C/EBPs). Hiroshi Ishikawa, Veysel Fencki, Erica E Marsh, Ping Yin, Debu Chakravarti, Serdar E Bulun.[*] *Division of Reproductive Biology Research, Dept of Ob/Gyn, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.*

Objective: Aromatase is the key enzyme that catalyzes the conversion of C19 steroids to estrogens in a number of human tissues including uterine fibroids. Aromatase expression in uterine fibroids is clinically significant since an aromatase inhibitor could shrink a fibroid in a perimenopausal woman. Although we demonstrated that the PGE2-responsive proximal promoters I.3 and II regulate aromatase expression in fibroid cells, the cis-acting elements in the promoter region or the transcriptional factors that bind to these elements are unknown. The objective is to define the molecular mechanism responsible for activation of aromatase promoter I.3 /II by PGE2 in fibroid cells.

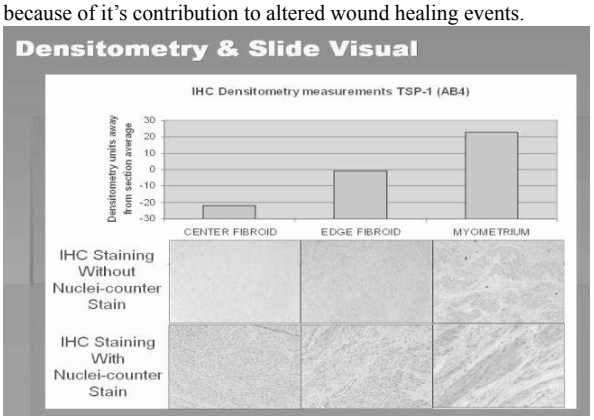
Methods: We used fibroid smooth muscle cells (n=15 patients) in primary culture, and performed aromatase enzyme activity assay employing radiolabeled substrate, then deletion and site-directed mutants of the promoter I.3 /IIregion fused to the luciferase reporter gene transfected to these cells.

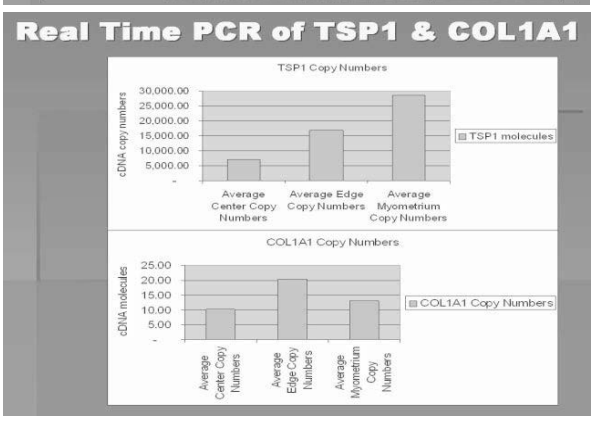
Results: PGE2 or a combination of dibutyryl-cAMP (Bt2-cAMP) plus phorbol 12, 13-diacetate (PDA) significantly stimulated aromatase enzyme activity in fibroid cells. Additionally, PGE2 or Bt2-cAMP plus PDA significantly

induced luciferase activity of -278, -517, -694, but not -100, -140 or -214/-17 bp promoter regions. These results indicate a critical region between -278 and -214 bp. In the -517/-17 region, we mutated 6 critical elements that potentially bind the transcription factors, such as CAATT/enhancer element binding protein (C/EBP)(3), Steroidogenic factor-1 /Liver receptor homolog-1 (SF-1/LRH-1)(2) and Activating transcriptional factor/cAMP responsive element binding protein (ATF/CREB) (1). Three C/EBP binding sites located at -350, -317, -245 and ATF/CREB site at -211 abolished responsiveness of the -517/-17 promoter region to both PGE2 and Bt2-cAMP plus PDA treatments.
Conclusions: Aromatase expression in uterine fibroid cells is regulated by multiple promoter elements in a complex fashion since mutation of each of the 3 C/EBP binding site consistently abolished PGE2 responsiveness. C/EBP isoforms may serve as major mediators for PGE2-dependent aromatase expression in uterine fibroids.

90

Thrombospondin-1 (TSP-1), a Multifunctional Glycoprotein Involved in Early Wound Healing, Is Inversely Correlated to COL1A1 Expression in Uterine Fibroids. Millie Behera,[1] Liping Feng,[1] Bryan Yonish,[1] William Catherino,[2] James Segars,[*3] Phyllis Leppert.[*1,3] *[1]Dept of Ob/Gyn, Duke University Medical Center, Durham, NC, USA; [2]Dept of Ob/Gyn, Uniformed Services University, Bethesda, MD, USA; [3]Reproductive Biology and Medicine Branch, NICHD, Bethesda, MD, USA.*
Uterine Fibroids are associated with many symptoms such as heavy uterine bleeding, painful intercourse, spontaneous abortion, rectal obstruction, and urinary obstruction which are associated with this type of benign tumor. In previous studies, we have demonstrated that fibroids are composed of altered, disoriented collagen fibrils and have presented evidence that fibroids may represent an arrested response to injury. To build on these results we focused our attention on TSP-1 (a molecule secreted by adult macrophages and monocytes upon injury), and COL1A1 (a gene that codes for fibrillar collagen found in most connective tissues). Our results show that in 10 subjects gene expression patterns for TSP-1 underexpress in both the edge and center of the fibroid, and COL1A1 was consistently and abundantly over-expressed in the center of the fibroid. These data were validated using multiple methods including microarray analysis using the Affymetrix U-133 platform, real time PCR, and finally visual localization of TSP-1 and COL1A1 using immunocytochemistry. Our findings suggest that the under-expression of TSP-1 contributes to the overall altered healing process leading to the formation of uterine fibroids and to the development of altered collagen fibrillogenesis and fibrosis. Further studies may elucidate TSP-1 as a possible therapeutic target to treat small fibroids because of it's contribution to altered wound healing events.





91

Ultrasonographic Mapping in the Preoperatory Evaluation of Uterine Myomas. Massimo Candiani, Stefano Izzo, Enrico Betto, Jennifer Riparini, Nicola Berlanda. (SPON: Anna Maria Marconi). *Obstetric & Gynecology, S Paolo Hospital, Milan, Italy.*
STUDY OBJECTIVE: To compare transvaginal ultrasonographic and intraoperatorive findings in patients with uterine myomas
METHODS: From January 2004 to September 2005, at our university hospital, 108 consecutive patients, undergoing myomectomy for symptomatic uterine myomas, were preoperatively investigated by transvaginal ultrasonography. The following data were collected: myoma's number, size, type and location. The patients were subsequently allocated into two group: 67 women with a total uterine volume ≤ 300 cc (1) and 41 >300 cc (2). Ultrasound was performed using Logos – Hitachi ultrasound machine equipped with a 5-9 MHz transvaginal probe. All ultrasound exams were performed by the same skilled physician. Preoperative data were compared to intraoperative findings.
RESULTS: Total number of removed myomas was 311, 139 in group 1 and 172 in group 2. The correspondence between US examination and surgical findings according to the number, dimension and location was 85.6%, 91.4% and 91.3%, respectivelly, in group 1 and 73.2%, 80.2% and 73.3%, respectivelly, in group 2.
The difference of the corrispondence between group 1 and group 2 was statistically significant (p=0.02, p=0.03 p=0.01, respectively)
CONCLUSIONS: The preoperative US scan evaluation of uterine myomas showed a lower sensitivity and a statistically significant difference of correspondence with surgical findings for uterine volumes >300cc. These results may have relevant implications in determining the surgical approach.

92

Use of GnRH-Agonists in Benign Metastatic Leiomyoma. Belinda J Yauger,[1] Jibri M Wiggins,[2] Elizabeth A Stewart,[*3] Mark Payson,[4] Alicia Y Armstrong.[1] *[1]Reproductive Medicine and Biology Branch, NICHD, NIH, Bethesda, MD, USA; [2]Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [3]Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA, USA; [4]Obstetrics and Gynecology, Walter Reed Army Medical Center, Washington, DC, USA.*
Hypothesis: Benign Metastatic Leiomyoma (BML) is a rare condition characterized by histologically benign leiomyoma present at distant sites. The gold standard for treatment is surgical resection, however, GnRH-agonist therapy may be preferable in pre-menopausal patients with multi-focal or non-operable disease.
Methods: The first patient is a 50 year old woman with a diagnosis of BML who presented with a new suprarenal mass, biopsy-consistent with leiomyoma, as well as musculoskeletal, lung, and pelvic masses. She was treated with six months of GnRH-agonist therapy. The second patient is a 37 year old woman with a diagnosis of BML who presented with shortness of breath and a new pleural effusion with mediastinal compression, as well as persistent lesions on the liver, diaphragm, and in the pelvis. She was started on GnRH-agonist therapy and followed with repeat radiological evaluation at six months of treatment.
Results: Six months of GnRH-agonist therapy produced an overall reduction in the size of lesions for the first patient. Treatment had a more varied effect in the second patient, with some lesions showing a slight increase and some a small decrease. The symptom of shortness of breath resolved with therapy.
Conclusions: GnRH-agonist therapy in BML can be beneficial in reducing size of lesions and symptoms due to these lesions. This therapy should be considered in patients who wish to avoid further surgery or for whom surgery is not an option.

93

Endogenous vs. Exogenous Estrogen Replacement (ERT) on Bladder Blood Flow (BBF) and ERα and ERβ Levels. Tova S Ablove,[1] Jason L Austin,[1] Terrance M Phernetton,[1] Ronald R Magness.[*1,2,3] *[1]Depts Ob/Gyn Perinatal Research Labs; [2]Anim Sci; [3]Peds, UW-Madison.*
The bladder is sensitive to fluctuations in ovarian hormones during the menstrual cycle and menopause possibly due to change in levels of estrogen receptors (ERs). Reproductive tract tissues are sensitive to vasodilatory effects of estrogen, the sensitivity of the bladder to these changes is unknown. **Objective**: Determine effects of endogenous ovarian or placental derived estrogen vs. ERT on BBF and the alterations in bladder ERα and ERβ levels. **Methods:** BBF was

measured using radio labeled microspheres in 33 ewes comparing BBF of Luteal (low estrogen), to Follicular (Preovulatory high estrogen) phase and Pregnant (high estrogen and progesterone). In oophorectomized (Ovx; 10-15 days post surgical menopause) ewes, we compared effects of acute (5ug/kg bolus @ 120 min) and prolonged (6ug/kg/day for 10 days) exogenous estradiol-17b to vehicle infusions. Bladders from 40 additional ewes matched to the above groups, ERα and ERβ levels were measured using western blot analysis. **Results**: BBF in intact sheep during the Luteal phase averaged 251± 90 (ml/min normalized per kg bladder weight) and was unaltered during the Follicular phase (185± 39), it was somewhat reduced in Pregnancy 120 ± 30 (P=0.055). Compared to Luteal phase sheep, Ovx raised bladder vascular resistance 2.9 fold from 567±201 to 1647±155 mmHg* min/ml and lowered BBF 72% from 251± 90 to 70 ml/min*kg (P<0.001). In Ovx animals, acute and chronic ERT lowered bladder vascular resistance (313±46 and 746±93 mmHg* min/ml) restoring BBF (350± 60 and 140± 20 ml/min*kg) to levels seen in ovary intact sheep (P>0.05). ERα predominated and ERβ was not detectable. In intact sheep, the phase of the cycle did not alter ERα levels, Pregnancy was associated with a 73% increase. When compared to the Luteal, Ovx, which lowered BBF, in contrast raised ERα concentrations 19%. ERT in Ovx sheep did not alter ERα levels. **Conclusion**: With Pregnancy and Ovx there were lower BBFs and elevated ERα levels, suggesting an inverse relationship between them in the bladder. The fall in BBF in pregnancy may be due to shunting of blood to reproductive tissues. Although ERT in Ovx sheep restored BBF it did not lower the elevated ERα levels back to Luteal controls. The rise in ERα levels in Pregnancy cannot merely be a response to reduced perfusion, but is likely due to the prolonged exposure to progesterone that occurs in pregnancy. *Support NIH HL49210, HD38843.*

94

The Effect of HOXA11 Upregulation on MMP-2 Expression in Mouse Fibroblasts: Implications in the Development of Pelvic Organ Prolapse. Kathleen A Connell, Heidi Chen, Vaagn Andikyan, Marsha K Guess, Hugh S Taylor.* *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: We have previously shown that the presence of HOXA11 is essential for the development of the murine uterosacral ligament and demonstrated decreased HOXA11 gene expression in women with pelvic organ prolapse (POP). MMP-2 and 9 influence extracellular matrix (ECM) remodeling by their ability to degrade the denatured fibrillar collagens I and III, which are two of the primary constituents of the uterosacral ligament. Previous studies have shown alterations in these enzymes in women with POP. The purpose of these experiments was to determine if overexpression of HOXA11 affects collagen I and III and/or matrix metalloproteinases 2 and 9.

Methods: NIH 3T3 mouse fibroblasts were cultured in DMEM containing 10% fetal bovine serum. After reaching 70% confluence, cells were transfected with a PTriX vector constitutively expressing HOXA11 or with the empty vector. After 24 hours, RNA was extracted and the expression of collagen Types I and III and MMP-2 and MMP 9 were evaluated using quantitative real time RT-PCR. Beta actin was used for normalization. All experiments were conducted in duplicate. Data was analyzed using Students *t* test.

Results: MMP-2 gene expression was significantly decreased in fibroblasts overexpressing the HOXA11 gene. After HOXA11 transfection MMP-2 expression decreased by 26% compared to controls treated with vector alone. (p<0.03) No significant differences were seen in collagen types I and III, or MMP-9 gene expression.

Conclusion: We have identified a novel pathway regulating collagen metabolism. HOXA11 regulation of MMP-2 expression contributes to the control of the ECM metabolism. Overexpression of the HOXA11 gene resulted in downregulation of MMP-2 gene expression in murine fibroblasts. Decreased expression of MMP-2 results in decreased collagen catabolism. Our findings suggest that altered ECM metabolism in women with POP may be due to downregualtion of the HOXA11/MMP-2 pathway. Targeted therapy using HOXA11 could potentially aid in the prevention or treatment of pelvic organ prolapse.

95

Can We Predict the Need for Cesarean Section To Avoid Severe Perineal Lacerations? Bela Kudish, Michael Kruger, Robert J Sokol.* *Obstetrics and Gynecology Dept, Wayne State University, Detroit, MI, USA.*

Objective: Conflicting opinions exist in both the literature and clinical care regarding the future role of elective cesarean section to avoid severe perineal lacerations. Presently, the key decisions that affect the rate of anal sphincter tears are made in the second stage of labor by implementing operative vaginal delivery and/or episiotomy. The purpose of this study is to develop an obstetric model of main antepartum pre-labor factors that allow for prediction of the development of anal sphincter trauma in the delivering patient.

Study design: Using a prospectively collected computerized perinatal database of a tertiary care institution, we examined antepartum maternal and obstetric factors, including maternal age (MA), ethnicity, nulliparity status (NS), parity, gestational age (GA), and estimated birth weight (EFW) for all singleton, vertex vaginal live births between 1996 and 2003. Based on β coefficients obtained from stepwise logistic regression (LR) of pre-labor risk determinants of severe perineal laceration, a model was developed for prediction of probability of developing anal sphincter tear. Robustness versus shrinkage of performance of the rule was examined by comparing receiver operator characteristic (ROC) curve for the predicted versus observed rates of severe perineal laceration.

Results: Over the 7-year study period, there were 33,564 vaginal deliveries meeting inclusion criteria. 3.65% of subjects experienced severe perineal trauma. LR yielded a rule in which MA (OR 1.04, 95%CI 1.03-1.05), African American ethnicity (OR 0.55, 95% CI 0.48-0.62), NS (OR 3.37, 95% CI 2.6-4.37), parity (OR 0.59, 95% CI 0.51-0.68), GA (OR 1.19, 95% CI 1.15-1.23), and EFW ≥4000 gm (OR 2.77, 95% CI 1.58-4.86) were significant pre-labor determinants of severe perineal laceration. The ROC curve for the predicted versus observed rates of severe perineal tear revealed predictive validity of the rule at a sensitivity of 80%, specificity of 66%, PPV of 8.2%, and NPV of 99%.

Conclusion: Even though the developed obstetric model allows to accurately predict women that would not develop severe perineal laceration, its ability to identify individuals that can potentially benefit from undergoing elective cesarean delivery to avoid severe perineal trauma is poor. More research is needed to identify additional pre-labor factors that can be included in this obstetric model before elective cesarean section can be offered as a solution.

96

Assessing Cervical Remodelling Prior To Induction of Labour by Electrical Impedance Spectroscopy: Correlation with Labour Characteristics and Outcomes. Dilly O Anumba,*[1] Soma Mukherjee,[1] Saurabh V Gandhi,[1] Brian H Brown.[2] *[1]Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom; [2]Biomedical Engineering, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

Objectives: Prelabour cervical ripening results from molecular events that include leucocyte infiltration, collagen metabolism, and altered hydration. Digital assessment of ripening has a high observer variation, and the value of cervical sonography is unclear. We sought to determine whether the electrical resistivity of the cervix prior to induction of labour may summarise cervical remodelling processes more accurately than clinical assessment, thus correlating better with labour outcomes.

Materials and Methods: Cervical electrical impedance (CI) and Bishop Score (BS) were determined in 83 women prior to induction of labour. CI was determined using a 9mm probe attached to the Sheffield v3.5 Impedance Meter. Data at 30 electrical frequencies were used to generate impedance spectra that conformed to the Cole Equation. We compared CI and BS for labour onset, duration, need for syntocinon augmentation and mode of delivery, employing parametric/nonparametric tests, as well as tests of correlation (Spearman's and Pearson's, cc) and prediction (area under the ROC curves, AUC).

Results: Cervical impedance at all frequencies showed a negative correlation with BS (P < 0.05). Low CI and high BS were highly predictive of multiparity (AUC P < 0.01 and < 0.05 respectively). Compared to BS, CI showed a better correlation with labour duration (cc: -0.17, P < 0.2 vs. cc 0.3, P < 0.01 respectively), and improved prediction of the need for syntocinon augmentation of labour [AUC (95% CI) 0.55 (0.42, 0.67) vs. 0.64 (0.52, 0.76) respectively].

The BS showed a strong correlation with the time to the onset of labour (cc -0.5 $P < 0.001$) whilst CI did not. Low BS was predictive of delivery by Caesarean Section for failure to progress (AUC 0.8 $P < 0.01$), a trend also noted for high CI and nulliparity ($P = 0.1$).

Conclusion: Pre-labour CI appears to predict long, as well as, dysfunctional, labours better than BS. We have recently shown that modifying the distance between the injecting and sensing electrodes alters the fraction of injected current that penetrates cervical stroma, and may enhance the clinical utility of this technique. Further work will clarify whether CI is complimentary or supplementary to traditional pre-induction assessment methods.

97

Urinary Myo-Inositol/Chiro-Inositol Ratios Efficiently Screen for Gestational Diabetes Mellitus. Brian J Koos.* *Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Testing glycemic responses to an oral glucose load is widely recognized as a suboptimal screen for gestational diabetes mellitus (GDM). Impaired conversion of *myo*-inositol (*myo*-I) to D-*chiro*-inositol (*chiro*-I), a putative mediator of insulin activity, may be involved in GDM. **Objective:** This study was designed to determine whether urinary inositol concentrations would be an efficient marker for GDM. **Methods:** The study comprised 25 normal gravidas (NG) and 21 women with GDM, who were matched by age, gestational age, body mass index, and race/ethnicity. Subjects were recruited after a 1-h, 50-g glucose test at 24-28 weeks of gestation and a subsequent 3-h, 100-g glucose challenge for those with screening plasma glucose levels ≥140 mg/dl. Urine concentrations of *myo*-I and *chiro*-I were measured by gas chromography-mass spectrometry. **Results:** Mean screening plasma glucose concentrations for normal and GDM were 119 ± 1.7 (SE) and 164 ± 2.3 mg/dl ($p < 10^{-4}$), respectively. Random urinary *myo*-I concentrations were 31.1 ± 7.8 and 23.0 ± 3.0 µg/ml ($p > 0.05$), respectively, for GD and GDM with corresponding *chiro*-I levels of 2.52 ± 1.1 and 0.15 ± 0.03 µg/ml ($p < 0.03$). Mean log *myo*-I/*chiro*-I concentration ratios were 1.25 ± 0.08 and 2.36 ± 0.08 ($p < 10^{-5}$) for NG (n=25) and GDM (n=21), respectively, with a ratio of 1.82 completely separating NG from GDM (100% observed sensitivity and specificity). Assuming a Gaussian distribution for this log ratio, these results predict a sensitivity and specificity for GDM of 93% and 91%, respectively. Paired log inositol concentration ratios for random and 24-h urine samples for 22 NG and 15 GDM, which ranged from 0.5 to 3.11, were virtually identical. **Conclusion:** Random urinary inositol concentration ratios appear to be a convenient and efficient screen for GDM.

Supported by the American Diabetes Association.

98

Artificial Neural Network: Control of Ultrasound Operator Measurement Errors. Filiberto Maria Severi,*[1] Caterina Bocchi,[1] G Cevenini,[2] F Filosomi,[1] F Calonaci,[1] P Barbini,[2] Felice Petraglia.*[1] *[1]Dpt of Pediatrics, Obstetrics and Reproductive Medicine; [2]Dpt of Surgery and Bioengineering, University of Siena, Siena, Italy.*

Objective

The fetal biometry used for sonographic estimation of fetal weight (EFW) is affected by human errors. We evaluated the performance of an Artificial Neural Network (ANN), we previously designed and tested, in enhancing the accuracy of fetal biometry detection by pointing out measurement errors and suggesting the operator to re-measure suspected wrong data.

Methods

61 fetuses were evaluated within 5 days of delivery by US parameters [BPD, HC, AC, FL, AF, ultrasound-delivery interval (US-D)]. Data were introduced into ANN to evaluate the probability of their correctness, the agreement among each fetal biometric parameter measured and a definitive EFW. The ANN performance was assessed both in its initial estimates and in those obtained after the re-measurement of suspected wrong data. For comparison we used the EFW derived by 182 formulas (from 59 published papers). Mean absolute error percent (MAE%) was calculated: differences were determined by using paired t-test.

Results

In 16 out of 61 cases biometry was measured once and in 45 cases it was repeated 2 or more times. US-D was 0.9±1.2 days, mean GA at delivery was 39±1.7 wks and mean fetal weight 3399±588 g. 18 formulas (from 7 papers) showed the same performance of ANN ($p > 0.05$). The correction of detected errors yielded statistically significant improvements (table) not only in ANN EFW (MAE% from 6.5 to 2.6) but also when the new biometry was tested by the 7 best models (i.e. Hadlock formula MAE% from 6.7 to 3.5).

Conclusions

We suggest a role of ANN as quality/control system for fetal biometry detection. Its use allows the biometric errors detection, analyzing in real time the congruity of each fetal parameter in respect to others, forcing the operator to repeat suspected wrong measurements till the percentage of congruity is high. Reducing human errors ANN enhances EFW and it can represent also an effective self-training tool for operators.

MAE% ± SD before (T1) and after (T2) ultrasound biometric corrections by ANN

	T1	T2	p value
ANN	6.5±4.5	2.6±2.6	<0.0001
Ott 86	5.7±4.2	4.3±2.8	0.0266
Combs 93	5.8±4.3	4.2±2.8	0.0086
Hill 85	6.2±4.4	4.6±2.5	0.0042
Woo 86	6.6±4.7	4.6±2.6	0.0021
Benson 87	6.7±4.5	4.9±2.9	0.0117
Hadlock 85	6.7±4.6	3.5±2.7	<0.0001
Robson 93	6.7±4.7	5.4±3.8	0.0464

99

Is Routine Sonographic Cervical Surveillance in Triplet Gestations Associated with a Decreased Incidence of Preterm Delivery? Charlotte Clock,[1] James T Kurtzman,[2] Michael Haydon,[1] Jennifer McNulty,[3] Vineet Shrivastava,[1] Manuel Porto.[1] (SPON: Deborah A Wing). *[1]Obstetrics and Gynecology, University of California, Irvine, Orange, CA, USA; [2]Maternal-Fetal Medicine, Saddleback Memorial Medical Center, Laguna Hills, CA, USA; [3]Maternal-Fetal Medicine, Long Beach Memorial Medical Center, Long Beach, CA, USA.*

OBJECTIVE: To determine if routine sonographic cervical length surveillence in triplet gestations is associated with a decreased incidence of preterm delivery.

STUDY DESIGN: This is a retrospective chart review of 115 triplet pregnancies delivering at Saddleback Memorial Medical Center, Long Beach Memorial Medical Center and the University of California-Irvine Medical Center between January 1998-August 2004. In 82 pregnancies, cervical length (CL) surveillance (≥2 CL measurements prior to 24 weeks' GA) was routinely used. These were compared to a control group of 33 pregnancies in which CL was not performed (no-CL). Patients with cerclage or indicated delivery (<32 weeks') were excluded. The primary outcome was gestational age (GA) at delivery.

RESULTS: There was a non-significant trend toward earlier GA at delivery between groups (CL:33.5±2.5 wks;no-CL:32.4±3.3 wks; p=0.046, CI [0.023-2.28]). However, a significantly higher proportion of subjects in the no-CL group delivered <28 weeks' GA. This difference was no longer significant when comparing the groups who delivered <32 weeks' GA.

Gestational Age at Delivery

	Cervical Length	No Cervical Length	P
Delivery <28 weeks'	2/82 (2.4%)	4/33 (12%)	0.035
Delivery <32 weeks'	19/82 (23.2%)	10/33 (30.3%)	0.426

CONCLUSION: While routine sonographic cervical length assessment in triplets is not associated with a significant prolongation of overall GA at delivery, our study demonstrates that regular cervical surveillance is associated with a significant decrease in extreme prematurity (<28 weeks' GA).

100

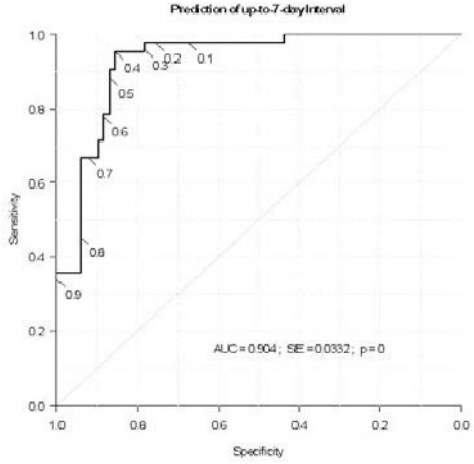
Predictors of Interval to Delivery Following Evaluation for Decreased Fetal Movement. Kimberly W Hickey,[1] Joy S Vink,[1] John C Pezzullo,[1] Nisha A Vyas,[1] Alessandro Ghidini,*[2] Sarah H Poggi.*[2] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, Inova Alexandria Hospital, Alexandria, VA, USA.*

Objective:

Decreased fetal movement has been associated with fetal demise, fetomaternal hemorrhage, and uteroplacental insufficiency. Evaluation typically includes nonstress test (NST) and amniotic fluid index (AFI) with expectant management of patients with normal results. Our objective was to identify risk factors for delivery at <7 days after reassuring standard testing for decreased fetal movement.

THURSDAY

Methods:
Ultrasound exams were performed on a cohort of women (n=106) at <40 weeks presenting with deceased fetal movement. Fetal growth, placentation, and presentation were assessed. Univariate and stepwise logistic regression analyses was performed to identify risk factors predicting delivery within 7 days of testing with p<0.05 considered significant.
Results: After taking into account gestational age (p<0.040), increasing maternal age (p< 0.014), female gender (p<0.026), and presence of obstetrical complications (p<0.000) were independently associated with interval to delivery <7 days in the presence of reactive NST and normal AFI. A formula incorporating the above variables allowed sensitivity of 80% with false positive rate of 12% for the prediction of delivery <7 days.A formula incorporating the above variables allowed sensitivity of 80% with a false positive rate of **% for the prediction of delivery <7 days.
Conclusions: In the presence of reassuring NST and AFI, patients with decreased fetal movement are more likely to be delivered within 7 days of testing in the presence of increasing maternal age, obstetrical complications and, female gender.



101

Can Clinical Factors Predict the Outcome of External Cephalic Version: A Meta-Analysis. Marjolein Kok,[1] Jeltsje Cnossen,[2] Joris van der Post,[2] Brent Opmeer,[3] Ben Willem Mol.[4] (SPON: Eric AP Steegers). *[1]Obstetrics & Gynecology, Academic Medical Centre, Amsterdam, Netherlands; [2]General Practice, Academic Medical Centre, Amsterdam, Netherlands; [3]Clinical Epidemiology & Biostatistics, Academic Medical Centre, Amsterdam, Netherlands; [4]Obstetrics & Gynecology, Maxima Medical Centre, Veldhoven, Netherlands.*
Objective: To systematically review the medical literature reporting on potential clinical prognosticators for the outcome of external cephalic version (ECV).
Data sources: Medline, Embase, Cochrane Library, manual searching of bibliographies of known primary and review articles.
Methods of study selection: Data from studies reporting on potential clinical prognosticators and ECV success rates that allowed construction of a 2x2 table were selected. Language restrictions were not applied. Two reviewers independently selected studies and extracted data on study characteristics, quality, and accuracy. Odds ratios were calculated and plotted in forest plots. When homogeneity could not be rejected we used a fixed effect model to calculate a common odds ratio and 95% confidence interval. When homogeneity was rejected (p<0.05) we used a random effect model.
Results: We detected 37 primary articles, with a total of 7372 women, that met the inclusion criteria. Multiparity (OR 3.5 (95% CI 3.1 – 4.0), non engagement (OR 9.2 (95% CI 6.3 – 13.5), uterine relaxation (OR 18.8 (95% CI 12.1 – 29.2), a palpable fetal head (OR 6.3 (95% CI 4.3-9.2) and maternal weight less than 65 kg (OR 1.8 (95% CI 1.2 – 2.6) were predictors for successful external cephalic version.
Conclusion: Multiparity, non engagement, uterine relaxation, a palpable fetal head and a maternal weight less than 65 kg are clinical predictors for successful ECV. This knowledge can be taken in consideration when counseling women for an ECV attempt.

Figure 1: Study s election process.

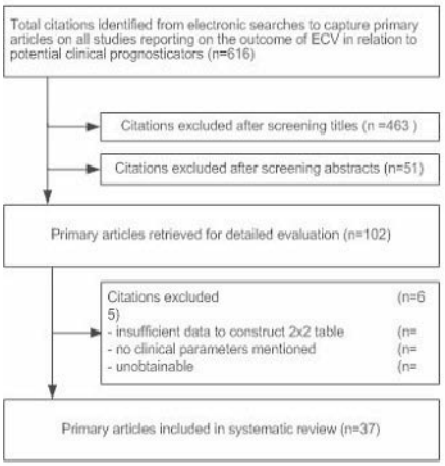
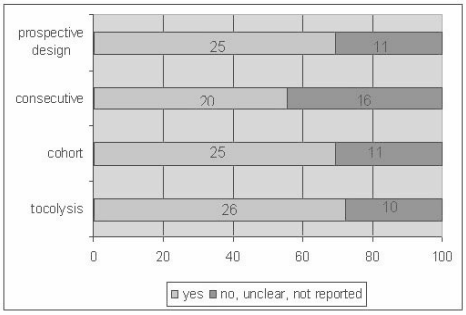


Figure 2: Methodological and reporting characteristics of studies included in the systematic review. Data presented as 100% stacked bars; figures in stack represent number of studies.



102

Contingent Voucher Based Incentives To Reduce Maternal Smoking Improve Fetal Growth. Ira M Bernstein,*[1] Laura Solomon,[2] Sarah Heil,[3] Mary Ellen Lynch,[3] Yamara Coutinho,[3] Colleen S Thomas,[4] Gary J Badger,[4] Stephen Higgins.[3] *[1]OB/GYN, Univ. of VT, Burlington, VT, USA; [2]Psychology, Univ. of VT, Burlington, VT, USA; [3]Psychiatry, Univ. of VT, Burlington, VT, USA; [4]Medical Biostats, Univ. of VT, Burlington, VT, USA.*
BACKGROUND: Maternal cigarette smoking during pregnancy is associated with reduced fetal growth and an increase in preterm birth. We sought to determine the effect of a contingent voucher based incentive program designed to reduce maternal smoking in pregnancy on fetal growth patterns.
METHODS: Fifty six women were studied who had been randomized in a prospective study to receive redeemable vouchers either contingent on evidence of smoking abstinence (Intervention group-IG, n=28) or independent of evidence of smoking abstinence (Control group CG, n=28). We performed ultrasound examinations at 30 and 34 weeks gestation for all subjects measuring BPD, HC, AC, FL and estimating fetal weight. We also recorded newborn birth weight and sex, maternal age, prepregnancy BMI, 1 hour glucose screening results, illicit drug exposure and parity. Statistical analyses were performed based on t-tests, Fisher's Exact test and stepwise regression with P<0.05 accepted for significance
RESULTS: We found no significant differences between groups with respect to maternal age, parity, 1 hour glucola, newborn sex or prepregnancy cigarette consumption. There was a tendency for the IG to have a lower prepregnancy BMI than the CG (IG 23.1 $\pm$ 5.3, CG 26.1 $\pm$ 6.7, p=0.07 and to have less frequent use of narcotics (IG = 0/28, CG = 5/28 (18 %), p=0.05). At the final antepartum assessment, there was significantly more smoking abstinence in the IG (11/28, 39%) compared to the CG (3/28, 11%) p=.03. Stepwise regression analysis, which controlled for significant covariates demonstrated that subjects assignment to contingent based vouchers had significantly greater growth in both fetal femur length (p=0.04) and in estimated fetal weight (p=0.02) between the two ultrasound examinations.
CONCLUSIONS: Fetal growth, represented by changes in femur length and estimated fetal weight is significantly greater in women randomized to a contingent based voucher than in those randomized to non-contingent vouchers as part of a program to promote smoking cessation in pregnancy. Supported by NIH RO1 DA 14028.

103

Non-Invasive Fetal Hemoglobin Monitoring. Donato D'Antona,[1] Guido Ambrosini,[1] Erich Cosmi,[1] Alessandra Andrisani,[1] Giovanni Battista Nardelli,[1] Giovanna Monegato,[1] Maurizio Clementi,[2] Felice Petraglia.[*3] *[1]Dipertimento di Scienze Ginecologiche e della Riproduzione Umana, University of Padua, Padua, Italy; [2]Genetica Clinica ed Epidemiologica Dipartimento Pediatria, University of Padua, Padua, Italy; [3]Pediatria, Ostetricia e Medicina della Riproduzione, University of Siena, Siena, Italy.*

Objective

Several conditions can lead to fetal anemia, such as red-cell alloimmunization or infections; fetal hemoglobin levels determination is necessary for a correct management and eventually for a therapeutic fetal transfusion.

Fetal hemoglobin levels have been evaluated with the use cordocentesis. Recently Mari elaborated a new algorithm to calculate the fetal hemoglobin value form the peak velocity of the middle cerebral artery with Doppler ultrasonography. More recently the DIAMOND Study group paper gives evidence that the Doppler ultrasonography can safely replace the invasive testing to predict fetal anemia comparing the observed values to the Mari's chart.

Methods

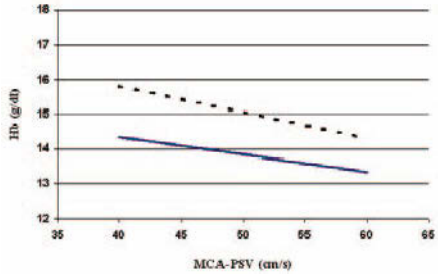
We have recently conducted a study to predict fetal anemia at term of pregnancy using the middle cerebral artery peak systolic velocity (MCA-PSV). We included only normal pregnancies in which a caesarian section has been programmed because of previous CS. Immediately after baby's birth the neonatal hemoglobin was obtained from the cord.

Results

The observed values were significantly different from those expected using the Mari's chart (solid line in fig.1). We have calculated a regression curve fitting our data (dotted line) and the result ($p<0.001$) is: Hb = 18,86 – 0,076 MCA-PSV

Conclusions

Our data suggests that Mari's chart is not applicable in the third trimester, probably due to the different viscosity of fetal blood. However, we further stress the usefulness of this non-invasive method also in late pregnancy. The prediction of fetal anemia may modify the delivery approach from the obstetricians.



104

Earlier Gestational Age at Ultrasound Evaluation Predicts Adverse Neonatal Outcomes in the Preterm AGA Fetus with Oligohydramnios. Joy Vink,[1] Kimberly Hickey,[1] Alessandro Ghidini,[*2] Shad Deering,[1] Adrian Mora,[2] Sarah Poggi.[*2] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA; [2]Obstetrics and Gynecology, Inova Alexandria Hospital, Alexandria, VA, USA.*

Objective: Oligohydramnios is related to adverse perinatal outcomes particularly when associated with fetal growth restriction (FGR). The purpose of this study was to delineate predictors of adverse perinatal outcomes in cases of preterm oligohydramnios associated with appropriate for gestational age (AGA) fetal biometry.

Study Design: A database of preterm AGA fetuses (<37 weeks) presenting for evaluation of oligohydramnios (defined as AFI <10th centile) in the third trimester with delivery information and uterine artery Doppler indices (average resistance index (RI) and bilateral notching) available was prospectively collected (n=90). Amniotic fluid index (AFI) and birth weight (BW) centiles were calculated using standard tables. Chi square and Student T-test were used to evaluate for predictors of adverse perinatal outcomes including BW ≤ 10th centile, stillbirth, NICU admission, 5 minute Apgar score < 7, placental abruption, preterm delivery (<35wks), and pre-eclampsia.

Results: Patients destined to experience poor perinatal outcomes (22%) were demographically similar to those experiencing normal outcomes in terms of maternal age (P=0.5), ethnicity (P=0.9), body mass index (P=0.3) and parity (P=0.9). However, at risk patients were more likely to present with oligohydramnios at an earlier GA than those not at risk (33.0 ± 3.0 vs. 34.4 ± 2.0 weeks; P = 0.02). There were no differences in perinatal outcomes associated with AFI centile (P=0.9), increased average uterine artery RI (P=0.5), bilateral notching (P=0.4) or a combination of increased uterine artery RI and bilateral notching (P=0.2).

Conclusion: Patients with preterm AGA fetuses who present with oligohydramnios at an earlier GA are at risk for adverse perinatal outcomes compared to those presenting later in gestation. Sonographic indices, particularly average uterine artery RI and bilateral notching, were not useful predictors of adverse outcomes.

105

3D and 4D Ultrasound: A Better Look in Fetal Echocardiographic Evaluation. Simcha Yagel,[*] Sarah M Cohen, Baruch Messing, Dan V Valsky. *Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*

Background: Late advances in the technology of 3D/4D ultrasound systems allow almost real-time 3D/4D fetal heart scans. It appears that 3D/4D ultrasound in fetal echocardiography can make a significant contribution to inter-disciplinary management team consultation, health delivery systems, parental counseling, and professional training. We aimed to evaluate the application of 3D/4D ultrasound in the diagnosis and management of fetal anatomic and functional heart disease.

Methods: We have applied 3D/4D ultrasound to the fetal echocardiography portion of our 13-16 and 21-24 gestational weeks fetal anomaly screening ultrasound scans since 2003. In addition to the standard 2D gray-scale and color Doppler scan, 3D/4D technologies were applied including 3D power Doppler (3DPD), spatio-temporal image correlation (STIC) acquisition, and B-flow, as well as 3D rendering and inversion mode (IM) applied during post-processing of stored volumes.

Results: We accrued >400 normal fetal echo exams including 3D/4D to establish a learning curve of these emerging technologies. We applied 3D rendering of STIC volumes to 136 normal fetuses and 35 cases of cardiac anomalies, evaluating the interventricular, interatrial, and coronal atrio-ventricular valves planes. B-flow was used to evaluate 199 normal fetuses and 11 cases of venous anomalies and 25 cases of great vessels malalignment. STIC acquisition was combined with IM to evaluate 100 fetuses to establish nomograms of right and left end-systolic and end-diastolic ventricular volumes; 100 additional cases of diagnosed congenital heart disease were evaluated with this modality; in 10 it was found to have added value in the overall evaluation of disordered cardiac function.

Conclusions: 3D/4D ultrasound in fetal echocardiography has added value in the diagnosis and evaluation of congenital heart defects and cardiac dysfunction.

106

Estimated Fetal Weight Limits the Usefulness of the SHOuLDER Score. Shobha H Mehta, Michael Kruger, Robert J Sokol.[*] *Obstetrics and Gynecology, Hutzel Hospital/Wayne State University, Detroit, MI, USA.*

Objective:

Management of patients suspected of increased risk of shoulder dystocia (SD) is hindered by poor predictability and the undesired increase in unnecessary cesarean deliveries (CD). We previously reported on the potential utility of the SHOuLDER score in reducing the prevalence of SD, including birth weight (BW) as a factor. Our objective is to compare the efficacy of the score using estimated fetal weight (EFW) instead.

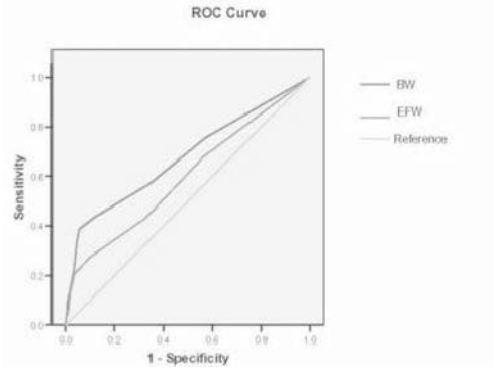
Materials and Methods:

Using ICD-9 codes and the hospital perinatal database all SD cases were identified between 1/96-1/01. Charts were reviewed and maternal demographic and labor data were collected. Controls consisted of those patients that delivered before and after each SD case, as identified by the database. Logistic regression was used as a guide to identifying those factors that were associated with SD outcome as well as the weight of each factor. These statistical analyses were used to assign points to each risk factor. The points were summed, and cutoff points were assessed capturing the highest number of SD cases with the lowest rate of false positives (controls with an elevated score).

Results:

207 SD cases occurred during the 5-year study period, to which 414 controls were assigned. Following statistical analysis, point assignments for risk factors were as follows: age >35 =1, diabetes = 3, maternal weight > 200 lbs = 1, maternal height < 5'2" = 2, history of prior SD = 2, estimated fetal weight >4000 g = 4, and 2nd stage of labor > 120 min =1. A cut-off of 5 points or greater was used to predict SD.

Conclusions:
SHOuLDER score (maternal Size, maternal Height, Old age, prolonged Labor, Diabetes, Estimated weight, and Recurrence) can be used to predict 16.4% of SD cases when using BW but only 9.9% of SD cases when using EFW, for the same specificity. To prevent 1 in 6 SD, 20 CD would need to be performed (rather than 12 as predicted using BW). The bottleneck is EFW; a protocol is needed to better predict it.



107

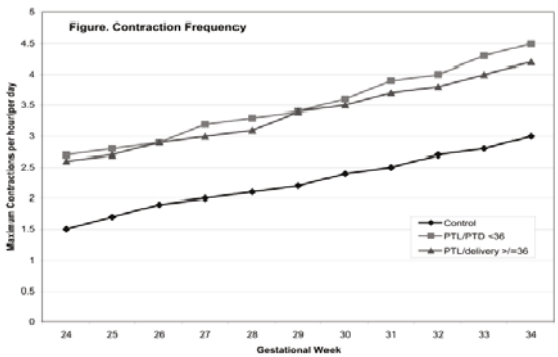
Antepartum Uterine Contraction Patterns in Twin Pregnancies with and without Preterm Labor and Delivering before or after 36 Weeks. Victor Hugo Gonzalez-Quintero,[1] Niki B Istwan,[2] Debbie J Rhea,[2] Lorna I Rodriguez,[1] Loren Smarkusky,[1] Jena Carter,[1] M Camille Hoffman,[1] Antoeneta Muller,[1] Amanda Cotter,[1] Gary J Stanziano.[2] (SPON: John C Morrison). *[1]Obstetrics and Gynecology, University of Miami, Miami, FL, USA; [2]Clinical Research, Matria Healthcare, Marietta, GA, USA.*

OBJECTIVE: To identify differences in antepartum uterine contraction patterns in twin pregnancies with and without preterm labor (PTL).

METHODS: Twin gestations enrolled for outpatient surveillance with twice daily electronic uterine activity surveillance and telephonic nursing assessment, without interventional delivery were identified. The maximum contraction frequency (MCF) per patient per day was determined for recordings obtained at 24-34.9 weeks' gestation. Mean MCF for each gestational week was compared between women without PTL or preterm delivery (PTD) <36 weeks (control group, n=28,832 monitored days) and those with a PTL diagnosis delivering at <36 weeks (PTL/PTD group, n=149,822 monitored days), and those with PTL with delivery ≥36 weeks (PTL/GAD ≥36 group, n=89,186 monitored days).

RESULTS: Data from 7891 patients with 267,840 monitored days were analyzed. 91.7% of twin pregnancies were complicated by PTL. 67.3% of patients with PTL delivered at <36 weeks. MCF at each gestational week was significantly higher for patients experiencing PTL with or without PTD compared to control (see figure, all $p<0.001$ by Mann-Whitney U). MCF was similar for patients with PTL with or without PTD <36.

CONCLUSIONS: Twin pregnancies complicated with PTL have a higher MCF than those pregnancies that do not experience PTL. Outpatient contraction surveillance may be beneficial in this population of high-risk pregnancies.



108

Betamethasone Induces Changes in the Relationship between Leptin and Adiponectin Levels in Pregnant Women. Emanuela Marinoni,[*] Francesca Ciardo, Giovanna Corona, Valentina Loguercio, Chrysula Zacharopoulou, Romolo Di Lorio.[*] *Dept. Gynecology, Perinatology and Child Health, University of Rome "La Sapienza", Rome, Italy.*

Objective: Leptin, adiponectin are adipokines derived from adipose tissue which play a role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues in both human and animals. In adult life, leptin is inversely related to adiponectin concentrations. Leptin and adiponectin are known to be produced within the intrauterine environment and have been suggested to be implicated in the regulation of placental growth, development and function and in fetal growth.Glucocorticoids were found to affect significantly adiponectin and leptin gene expression and secretion both in vitro and in animal models. In the present study we evaluated the effect of maternal administration of betamethasone on the correlation between adiponectin and leptin levels in maternal circulation.

Material and methods: betamethasone (12mg x 2 24hours apart) was administered to 21 pregnant women at risk of preterm delivery between 24 and 34 weeks of gestation, with blood samples taken at -10min, 3, 6, 12, 24 hours after the first dose and 12, 24 hours and 1 week after the second dose. Adiponectin and leptin concentrations were assayed by ELISA.

Results: Adiponectin was inversely correlated to leptin in basal samples (correlation coefficient = -0.646; $p<0.01$). Neither adiponectin nor leptin correlate with gestational age, maternal age or maternal BMI. After betamethasone administration the correlation between adiponectin and leptin was progressively reduced because of the progressive increase in leptin ($p<0.05$) levels up to 24 hours after administration. In samples collected at 12h after betamethasone this correlation was completely lost and was not restored after 1 week.

Conclusion: Glucorticoids in human pregnancy induce a perturbation in the relationship between leptin and adiponectin levels that is maintained after betamethasone administration is discontinued. This effect may be exerted on adipokines release by the placenta and may induce a permanent change in the intrauterine metabolic environment.

109

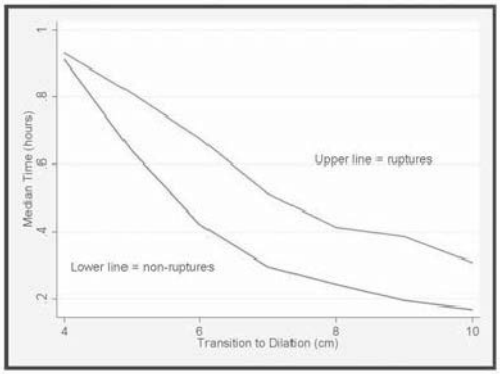
The Course of Labor in Women with Uterine Rupture Compared to Those without Rupture. George A Macones,[*1] David M Stamilio,[1] Jeffrey Peipert,[*1] Alison G Cahill,[1] Anthony Odibo,[1] Sarah J Ratcliffe,[2] Erika Stevens.[1] *[1]OB/GYN, Washington University in St Louis, St. Louis, MO, USA; [2]Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: To compare labor curves of women attempting VBAC who ultimately have a uterine rupture to those who do not rupture.

Study Design: This is a planned secondary analysis of a multicenter case-control of uterine rupture in women attempting VBAC. Cases of rupture and non-cases were identified by review of inpatient medical records. Data on the course of labor in cases and controls was collected in 15 minute increments, including information on oxytocin dose, cervical examination, and other information. An interval-censored regression model with a log normal distribution was fitted to estimate the transition times from one level of cervical dilation to the next, and to generate and compare labor curves for cases and controls.

Results: We identified 134 cases of uterine rupture and 670 controls. The duration of labor was slower for cases of rupture compared to controls, especially early in the active phase (2-4 cms) and late in the active phase (7-10 cms). There was a significant difference in the slope of labor curves between cases of ruptures (upper line- figure) and controls (lower line; $p=0.01$).

Conclusions: The course of labor in women who ultimately rupture is significantly slower than in women who do not.



110

Pregnancy Outcome in Lupus Pregnant Women with Antiphospholipid Antibodies: Effect of Treatment with Aspirin Plus Low Molecular Weight Heparin. Federico Mecacci,[1] Barbara Bianchi,[1] Riccardo Cioni,[1] Laura Giorgi,[1] Alessandra Moretti,[1] Carlotta Buzzoni,[1] Mauro Marchionni,[1] Michael Paidas.[3] (SPON: Graciela Krikun). *[1]Obstetrics and Gynecology, Careggi University Hospital, Florence, Italy; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

OBJECTIVE: During the last two decades, there has been an increasing understanding of reciprocal effects of pregnancy and systemic lupus erythematosus (SLE). Antiphospholipid antibodies (APA) are found in a significant proportion of lupus pregnant patients, and their presence has been associated with a variety of obstetric complications. Treatment has usually been reserved for the subgroup of lupus patients with antiphospolipid antibody syndrome (APS). There is no concensus regarding treatment of lupus patients with APA only.This study was undertaken to evaluate maternal and fetal outcomes in pregnant women with SLE and APA treated with aspirin and heparin therapy.

STUDY DESIGN: We prospectively studied a total of 50 pregnancies in 46 patients with SLE, 22 with APA, of whom 4 (18%) had APS; 24 of the 46 (52%) were APA-negative. Women were considered positive for APA if lupus anticoagulant and/or APA were detected in two or more occasions at least 6 weeks apart. APA-positive women received ASA (100mg/day) and a prophylactic dose of low molecular weight heparin (LMWH; dalteparin 5,000 IU/day s.c.). At onset of pregnancy, 59% of the APA women and 54% of APA negative patients were taking steroids with an average prednisone dosage of 7.8 mg/day (range 5-20).

No patient had history of previous thromboembolism. Pregnancy complications (spontaneous abortion, preeclampsia, preterm labour, IUGR, lupus flare, and therapeutic termination of pregnancy for flare), as well as neonatal outcome (birth weight, Apgar index and ponderal index) were compared between the two groups.

RESULTS No significant differences between the two groups were found with respect to the parameters of maternal and fetal-neonatal outocome considered, although the distribution of some complications was skewed (of a total of 18 flares, 12 were in APA-positive and 6 in APA-negative women; the only 2 cases of HELLP syndrome were observed in the APA-positive group).

CONCLUSIONS In SLE pregnant women with APA, treatment with low dose aspirin and LMWH can lower the incidence of maternal and fetal/neonatal complications to the rate observed in APA-negative patients.

111

Pregnancy Outcome after Stillbirth: The Effect of Low Molecular Weight Heparin in Thrombophilic and in Non-Thrombophilic Patients. Federico Mecacci,[1] Francesca Castiglione,[2] Barbara Bianchi,[1] Laura Giorgi,[1] Annalisa Pieralli,[2] Gian Luigi Taddei,[2] Carlotta Buzzoni,[1] Michael Paidas.[3] (SPON: Charles J Lockwood). *[1]Obstetrics and Gynecology, Careggi University Hospital, Florence, Italy; [2]Department of Human Pathology and Oncology, University of Florence, Florence, Italy; [3]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background and Objective: Stillbirth (SB) complicates 5-7 per 1,000 pregnancies with a significant recurrence risk. Women with thrombophilia have an increased risk of stillbirth. Primary aims of this observational study were: 1) verify the perinatal outcome of pregnancies following SB in patients with placental histopathology suggestive of maternal vascular disease (MVD); 2) compare perinatal outcome of these patients, termed thrombophilic- like (TL) with the outcome of patients with known thrombophilic disorders, termed KT, when both were treated with prophylactic low molecular weight heparin (LMWH).

Methods: This study was conducted at a single institution (Careggi Hospital, University of Florence). There were 105 patients with placental pathology from antecedent SB: 57 patients (54.2%) were KT and 48 patients (45.8%) were TL. Acquired and inherited thrombophilic evaluations were performed preconceptually. Placentas from the antecedent pregnancy were systematically assessed by two pathologists for the presence of MVD. For the index pregnancy, maternal and fetal outcomes were compared [fetal loss, intrauterine growth restriction (IUGR)preeclampsia, preterm delivery, abruption].

Results: There were no SB in either group. The composite risk of perinatal complications was 3 times greater in the KT group compared to the TL (70% vs 50%, O.R.=2.35, 95CI 1.06-5.24, pval .036). IUGR occurred in 21 % of the KT cases (O.R.=3.59). Birthweight was significantly less in the KT compared to the TL group(2865g vs 3151g, P=0.03).There were no serious complications associated with LMWH.

Conclusion: LMWH prevents SB in women at high risk for recurrent SB, irrespective of thrombophilia status. Patients with KT are at higher risk for pregnancy complications, in women with antecedent SB, despite prophylactic anticoagulation. A randomized trial is needed to assess utility and safety of prophylactic antecoagulation in women with antecedent SB and placental pathology suggestive of MVD.

112

Pregnancies Complicated by Fetal Congenital Heart Defect: Are the Obstetrical Outcomes Different? Jennifer Merriman,[1] Michal Elovitz,[*1] Samuel Parry,[*1] Stephanie Sober,[1] Jack Rychik,[2] Emmanuelle Pare.[1] *[1]Obstetrics & Gynecology, University of Pennsylvania, Philadelphia, PA, USA; [2]The Cardiac Center, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [3]Philadelphia, PA, USA.*

OBJECTIVE: Elective induction of labor (IOL) has been proposed to prevent unplanned deliveries in women carrying fetuses with congenital heart defects (CHD). However, elective IOL has been associated with higher cesarean section (CS) rates. We sought to determine if IOL increased the CS rate in women carrying fetuses with CHD.

METHODS: Women carrying a fetus with CHD confirmed by fetal echocardiogram who delivered in a single referral center in the past 5 years were identified using a perinatal database and ICD codes. Medical records were abstracted for demographics, medical/obstetrical history, and delivery outcomes. Women who had an elective IOL due to CHD were compared to those who had spontaneous labor. Bivariate and stratified analyses were performed using chi-square, Student t test and Mantel-Haenszel.

RESULTS: Of the 285 women identified, 207 (73%) women were eligible for and attempted vaginal delivery: 32 (15%) had IOL for other indications, 119 (57%) had IOL only due to CHD and 56 (27%) had spontaneous labor. Baseline characteristics and CS rate of the 2 study groups are shown in Table 1. The CS rate was similar in both groups (14% vs 13%), Primiparas were less likely to have elective IOL (NS), but had higher CS rate than multiparas. IOL did not increase the risk of CS for primiparas (RR=0.86; 95%CI 0.34-2.16).

CONCLUSION: In this population of women carrying fetuses with CHD, the rate of elective IOL was high, but contrary to what has been observed in different populations, elective IOL did not seem to increase the risk of CS, even for primiparas. Further analyses are planned to explore our results and determine if there is something intrinsically different about these women.

Table 1: Baseline characteristics and CS rates

	Spontaneous labor (n=56)	Elective IOL (n=119)	p value
Maternal age (mean±SD)	27.8±6.5 yrs	29.9±6.2 yrs	0.04
Gest age at delivery (mean±SD)	37.5±2.3 wks	38.8±1.1 wks	< 0.01
Birthweight (mean±SD)	2927±691g	3128±517g	0.06
Cervical dilation on admission (median)	3 cm	2 cm	< 0.01
CS rate	8 (14.3%)	16 (13.4%)	0.88

Table 2: Primiparas vs multiparas

	Prime (n=77)	Multip (n=98)	RR (95%CI)
Elective IOL	49 (63.6%)	70 (71.4%)	0.89 (0.72-1.10)
CS rate	15 (19.2%)	9 (9.2%)	2.12 (0.98–4.58)

113

Minor Trauma in Pregnancy: Is There an Increased Risk of Abruption? Alison G Cahill,[1] Jamie A Bastek,[2] David M Stamilio,[1] Anthony O Odibo,[1] George A Macones.[*1] *[1]Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO, USA; [2]Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, PA, USA.*

Objective: To define the incidence of and risk factors for abruption and adverse pregnancy outcome after minor trauma in pregnancy.

Methods: This prospective cohort study of patients presenting at or beyond 24 0/7 weeks gestation to a tertiary care center after non-catastrophic trauma was conducted over a 4 year period. Data was collected on maternal demographics, medical and obstetrical history, trauma mechanism and evaluation, and pregnancy outcome. Evaluation encompassed physical exam, lab tests including Kleihaur-Betke (KB), and fetal monitoring. Patients were monitored for a minimum of 4 hours and a maximum of 24 hours. If discharged, data was again collected at delivery. The primary outcomes were placental abruption and a composite pregnancy morbidity outcome defined as at least one of: delivery prior to 37 weeks, birth weight < 10%ile, or placental abruption. Univariate and multivariable analyses were performed.

Results: Of 317 patients evaluated for minor trauma, only 9 had a positive KB test (2.8%), and 2 had a fibrinogen <200 (0.6%). Delivery information was available on 256 patients (80.8%). There were no fetal or perinatal deaths, and 1 placental abruption which occurred at 41 weeks, 4 weeks after the evaluation

for a fall, with a KB of 0.00%. The composite outcome occurred in 49 cases (19.4%), and could not be predicted by the mechanism of trauma, a positive KB, fibrinogen <200, abdominal pain, uterine contractions, placental location or a combination of factors (Table 1).
Conclusions: The risk of placental abruption and adverse pregnancy outcome attributable to minor trauma is small and cannot be predicted with the clinical and laboratory factors commonly used. These data suggest that minor abdominal trauma in pregnancy does not require admission or laboratory evaluation. The extensive and time-consuming evaluations that occur after such events should be reconsidered.

Risk for and prediction of composite pregnancy outcome

Predictor variable	Unadjusted RR	95% CI	Sensitivity	Specificity
Positive KB	2.12	0.70 - 6.42	4.1 %	98.5 %
Fibrinogen < 200	2.64	0.64 - 10.78	2.0 %	99.5 %
> 5 Contractions/hr	0.71	0.42 - 1.19	41.7 %	47.8 %
Abdominal pain	1.04	0.46 - 2.39	10.2 %	90.3 %
Direct abdominal trauma	1.32	0.70 - 2.50	18.4 %	86.4 %

114

Neonatal Outcomes of Normotensive Pregnancies and Pregnancies with Preeclampsia or Gestational Hypertension at Term. Mounira Habli,[1] Baha Sibai,[*1] Richard Levine,[2] Cong Qian.[2] *[1]Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA; [2]Epidemiology, National Institutes of Health (NIH), Bethesda, MD, USA.*
Our objective was to compare neonatal outcomes of normotensive pregnancies (NT) with those of pregnancies with preeclampsia or gestational hypertension (PE/GH) delivered at 38, 39 and 40 weeks(wks).
Study design:
Secondary analysis of neonatal outcomes of infants born between $38^{0/7}$ and $40^{6/7}$ wks to healthy nulliparous women enrolled in a multicenter NICHD study. Neonatal outcomes were rate of NICU admission, duration of neonatal hospitalization and neonatal complications. We compared neonatal outcomes separately in infants from pregnancies with PE/GH and NT pregnancies delivered at 38, 39, and 40 wks. For each gestational age at delivery we compared neonatal outcomes from PE/GH and NT pregnancies stratified by spontaneous labor vs induced labor or section. Data were analyzed using t-test and chi square with P-value < 0.05 considered significant.
Results:
Neonatal outcomes were examined from a total of 2061 normotensive and 631 hypertensive pregnancies. Rates of NICU admission and respiratory support (oxygen treatment, CPAP, mechanical ventilation) were greater for the infants of women with PE/GH than the infants of NT pregnancies (table). Total neonatal stay was longer for infants of PE/GH pregnancies born at 39 wks and 40 wks. There were no differences in rates of SGA or RDS within each gestational age week of delivery. At 38 and 39 wks rates of respiratory support were greater for spontaneously delivered infants of women with PE/GH than in the NT pregnancies (data not shown).
Conclusion:
Rates of NICU admission and respiratory support were greater among infants born to hypertensive women at 38, 39, and 40 wks. This suggests the need for revision of the conservative management of preeclampsia beyond 37 wks.

	NT (N=419)	PE/GH (N=132)	NT (N=767)	PE/GH (N=216)	NT (N=875)	PE/GH (N=283)
GA(wks)	38	38	39	39	40	40
SGA#(%)	48(11.5)	13(9.9)	84(11.0)	23(10.7)	62(7.1)	30(10.6)
NICU admission#(%)	31(7.4)	19(14.5)*	64(8.3)	30(13.9)*	70(8.0)	57(20.1)***
Days in NICU (mean ± SD) (d)	5.7±5.5	3.8±2.9	3.7±3.4	6.1±7.5*	4.7±5.2	5.8±6.7
Total neonatal stay (mean ± SD) (d)	2.2±2.7	2.7±1.8	1.9±2.1	3.2±3.5***	2.1±2.4	3.4±3.6***
RDS#(%)	8(1.9)	2(1.5)	12(1.6)	7(3.2)	17(2.0)	9(3.2)
Respiratory support#(%)	32(7.7)	25(19.1)***	62(8.1)	38(17.6)***	76(8.7)	43(15.2)**

*P<0.05 **P<0.01***P<0.001 for the comparison of PE/GH with NT pregnancy

115

The MFMU Cesarean Registry: Combined Twin Delivery. James M Alexander.[*] *for the NICHD MFMU Network, Bethesda, MD, USA.*
Objective: To examine maternal and infant outcomes after a vaginal delivery of twin A and a cesarean delivery of twin B.
Methods: Between January 1, 1999 and December 31, 2000, a prospective cohort study of all cesareans was conducted at 13 university centers. This secondary analysis was limited to women with twin gestations who experienced labor and underwent cesarean delivery. We compared maternal and neonatal outcomes in women who had vaginal delivery of twin A and a cesarean delivery of twin B to those who had cesarean delivery of both twins.
Results: 1,028 women with twins experienced labor and underwent cesarean delivery, 179 (17%) had a combined vaginal/cesarean delivery. The women in the combined group were found significantly more often to have been multiparous and African American. Gestational age at delivery was similar in both groups (34.6 weeks vs. 34.6 weeks, p = 0.97). Selected maternal and infant outcomes are shown in the table.

	Combined n = 179 (%)	Both cesarean n = 849 (%)	OR 95% CI
Maternal:	7 (3.9)	40 (4.7)	0.82 (0.36 – 1.87)
Chorioamnionitis	23 (12.8)	70 (8.2)	1.64 (0.99 – 2.71)
Endometritis	2 (1.1)	7 (0.8)	1.36 (0.28 – 6.60)
Wound infection			
Infant:*	7 (4.0)	27 (3.2)	1.27 (0.54 – 2.96)
5-minute Apgar ≤ 3	0 (0)	2 (0.24)	–
HIE**	2 (1.2)	15 (1.8)	0.64 (0.15 – 2.84)
Confirmed seizures	4 (2.4)	32 (3.9)	0.60 (0.21 – 1.72)
Neonatal death	15 (8.5)	40 (4.8)	1.85 (0.99 – 3.42)
Proven sepsis			

* Data shown for twin B
**HIE = hypoxic ischemic encephalopathy

Conclusion: Combined delivery did not affect maternal outcomes related to infection or neonatal outcomes of the second twin when compared to twins where both were delivered by cesarean.

116

Antithrombotic Prophylaxis in Multiparous Women with Preeclamsia or Intrauterine Growth Retardation in Antecedent Pregnancy. Gabriele Urban,[1] Patrizia Vergani,[1] Roberta Tironi,[1] Patrizia Ceruti,[1] Emanuela Vertemati,[1] Francesca Sala,[1] Enrico Pogliani,[2] Elizabeth Triche,[3] Charles J Lockwood,[*4] Michael J Paidas.[4] *[1]Obstetric and Gynaecology, University of Milano-Bicocca, Monza, Italy; [2]Hematology, University of Milano-Bicocca, Monza, Italy; [3]Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, USA; [4]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
OBJECTIVE: To determine whether antenatal administration of low dose aspirin alone or in combination with low-molecular-weight-heparin reduces the recurrence of adverse pregnancy outcome (APO).
STUDY DESIGN: In this retrospective cohort study, a total of 84 consecutive multiparous with a previous history of severe preeclampsia (sPE), HELLP syndrome, intrauterine growth restriction (IUGR) were tested for acquired and inherited thrombophilias prior to the index pregnancy. Upon subsequent pregnancy, patients were assigned to receive one of three management strategies for the index pregnancy according to the preference one of the three attending physicians. Odds ratios of the association between treatment category and pregnancy outcomes were calculated from logistic regression models.
RESULTS: Of the 84 patients with antecedent sPE and/or IUGR (n=84) of 86 pregnancy, 29 women (33.7%) developed a subsequent APO. Forty eight (55.8%) women received LDA, 21 (24.4%) women LMWH plus LDA treatment daily, and 16 (18.6%) remained untreated during pregnancy. women combined treatment significantly reduced the risk of developing IUGR in the index pregnancy (OR=0.16, 95% CI: 0.03-0.98), and any APO (OR=0.18, CI: 0.04-0.96). Among women with antecedent sPE (n=52), combined treatment reduced APO in the index pregnancy (OR=0.04,CI: 0.01-0.96), IUGR (OR=0.02,CI: <0.01-0.46), and IUGR/sPE (OR=0.08,CI: 0.01-0.96).
CONCLUSION: Combined treatment with LDA and LMWH is strongly protective against the development of APO in a cohort of women with antecedent sPE and/or IUGR at high risk of recurrence. A randomized placebo trial should confirm this finding.

117

The Effect of Maternal Thrombophilia on Abruption Histology. Wendy Kinzler,[*1] Vinay Prasad,[2] Denise Elsasser,[1] Cande Ananth.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA; [2]Pathology, University of Arkansas Children's Hospital, Little Rock, AR, USA.*
BACKGROUND: Although the association between maternal thrombophilia and abruption has been established, it is not known whether differences exist among abruptions based on thrombophilia status.
OBJECTIVE: To determine if the histology of placental abruption differs when a maternal thrombophilia is present.
STUDY DESIGN: This was a multicenter, case-control study of women with abruption and delivering at ≥20 weeks' gestation, collected as part of the ongoing New Jersey Placental Abruption Study. Women were identified by clinical and pathological criteria of abruption. Maternal blood was collected postpartum and tested for protein S, protein C, activated protein C resistance ratio, antithrombin III, Factor V Leiden, prothrombin gene mutation, and

anticardiolipin antibodies. Cases were comprised of women with an abruption and a positive thrombophilia screen. Controls were comprised of women with an abruption and a negative thrombophilia screen. All placental histology was systematically reviewed by two perinatal pathologists, blinded to the abruption status. Comparisons of histological lesions were made between cases and controls based on Fisher's exact probability test, and P<0.05 was considered to denote statistical significance.

RESULTS: A total of 130 women with abruption were identified, of which eighty-five (65.4%) had at least one thrombophilia. Acute/chronic deciduitis (25% versus 58.1%, P=0.002) and villous hemorrhage (1.3% versus 12.5%, P=0.02) were less common among women with than without thrombophilia, whereas perivillous fibrin deposition was more common in women with thrombophilia (9.4% versus 0%, P=0.05). There was no difference in the overall presence of infarcts between the 2 groups (23.5% versus 37.8%, P=0.10). However, when an infarct was present (28.5% of abruptions), the presence of an old infarct was more common among women with a thrombophilia (90% versus 41.2%, P=0.004).

CONCLUSION: Abruption with maternal thrombophilia is associated with higher rates of old placental infarcts and perivillous fibrin deposition, but lower rates of deciduitis and villous hemorrhage, compared with abruption without thrombophilia. This suggests a longstanding disruption of placental blood flow in abruption cases associated with thrombophilia.

118

Thromboprophylaxis in Women with Inherited Thrombophilias and No Prior Thrombosis. Jennifer Warren, Robert M Silver,* D Ware Branch, T Flint Porter. *Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA.*

Objective: Thrombophilias have been associated with obstetric complications characterized by placental insufficiency including pregnancy loss, preeclampsia, placental abruption, and intrauterine growth restriction (IUGR). This has prompted many clinicians to screen and treat pregnant women for thrombophilias. However, heritable thrombophilias are often present in women with uncomplicated pregnancies and most available data comes from retrospective studies using self controls. Our objective was to evaluate the effect of thromboprophylaxis on pregnancy outcomes in women with inherited thrombophilias.

Methods: This is a retrospective cohort study of women with thrombophilias but no prior thrombosis. Medical records were reviewed for pregnancy outcomes, adverse events, diagnosis of thrombophilias, and management in subsequent pregnancies. Chi square and Fisher's exact tests were used to compare women who were and were not treated with thromboprophylaxis.

Results: Fifty-two women had 74 pregnancies subsequent to their diagnosis of thrombophilia. The diagnosed thrombophilias included Factor V Leiden (n=40), PT 20210A gene mutation (n=5), FVL/PT gene compound heterozygote (n=1), protein C and S deficiencies (n=5), and antithrombin III deficiency (n=1). Thirty-six (49 %) pregnancies were treated with heparin, 7 (9%) with low dose aspirin, and 31 (42%) were not treated. Women treated with heparin had similar rates of live births (74% vs. 77%), pregnancy loss (12% vs. 16%), preeclampsia (0% vs. 6%), abruption (5% vs. 0%), and IUGR (0% vs. 0%) as those not treated.

Conclusions: Pregnancy outcomes are often good in women with thrombophilias in the absence of treatment. Treatment of women during pregnancy with thrombophilias and no prior thrombosis should be considered investigational.

119

Fetal Thrombophilia Is Associated with Intrauterine Growth Restriction. P Pileri,[1] F Franchi,[2] A Martinelli,[1] S Calabrese,[1] G Frey,[1] E Biguzzi,[2] G Pardi,[1] Irene Cetin.*[1] *[1]IRCCS Policlinico Mangiagalli Regina Elena, Inst. of Obst. and Gynec, Milan, Italy; [2]IRCCS Policlinico Mangiagalli Regina Elena, Haemoph. and Thromb. Centre, Italy.*

Objectives. Thrombophilia is defined as a tendency to develop venous thrombosis. Some pregnancy complications, such as intrauterine growth restriction (IUGR), pregnancy induced hypertension (PIH) and preeclampsia (PE), are associated with an abnormal placental development, potentially related to disorders of the utero-placental circulation. A case-control study was performed in order to establish whether the presence of maternal and/or fetal factor V and prothrombin mutations are associated with pregnancy complications such as IUGR, PIH and PE.

Methods. IUGR were included if there was reduced intrauterine growth (U.S. measurement of abdominal circumference (AC) below the 10° percentile of reference values, alternatively a percentile reduction>40%, compared to the previous measurement of AC) and birth weight below the10° percentile. PIH was defined as a blood pressure >140/90 on 2 or more occasions occurring after 20 weeks of gestation without proteinuria. PE was defined as PIH with proteinuria (>0.3gr/24h). Controls were normal term pregnancies carrying appropriate for gestational age fetuses. Exclusion criteria were: multiple pregnancies, non Caucasian women, fetal malformations or chromosomal abnormalities. 104 cases (C) and 204 controls (N) were enrolled and tested for thrombophilia (factor V Leiden and prothrombin G20210A). Cases were classified as follows: 51 PE or PIH, 53 IUGR (20 with PE or PIH, 33 IUGR only).

Results. Thrombophilia was present in 13/204 (6.4%) mothers and in 19/204 (9.3%) fetuses of the controls compared to 8/104 (7.7%) mothers and 15/104 (14.4%) fetuses of the cases; the OR was 0.8 (CI 95% 0.3-2) and 2.4 (CI 95% 1-5.25) for mothers and fetuses, respectively. Table 1 presents our results in the different pathology groups.

Conclusions. Our results suggest that fetal thrombophilia is associated with IUGR, possibly due to inadequate placental development on the fetal side.

Groups	Mothers			Fetuses		
	N	C	OR (CI 95%)	N	C	OR (CI 95%)
PIH or PE (n=51)	13/204 (6.4%)	3/51 (5.9%)	0.9 (0.25-3.35)	19/204 (9.3%)	6/51 (11.76%)	1.09 (0.4-2.9)
IUGR with and without PE/PIH (n=53)	13/204 (6.4%)	5/53 (9.4%)	1.53 (0.52-4.5)	19/204 (9.3%)	9/53 (16.9%)	1.83 (0.84-4.7)
IUGR without PE/PIH (n=33)	13/204 (6.4%)	4/33 (12.1%)	2 (0.6-6.64)	19/204 (9.3%)	8/33 (24.24%)	3.12* (1.23-7.86)

120

Preterm Labor and Preterm PROM Are Characterized by an Increase in Maternal Serum Concentration of Galectin-1. Nandor G Than,[1] Offer Erez,[1] Derek E Wildman,[1,2] Samuel S Edwin,[1] Shali Mazaki-Tovi,[3] Francesca Gotsch,[1] Enola Cushenberry,[1] Beth Pineles,[1] Daniel Montegro,[1] Jimmy Espinoza,[3] Chong-Jai Kim,[4] Sonia S Hassan,[1,2] Zoltan Papp,[5] Roberto Romero.*[1,2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Molecular Medicine & Genetics, Wayne State Univ, Detroit, MI, USA; [3]Dept of Obstetrics & Gynecology, Wayne State Univ, Detroit, MI, USA; [4]Dept of Pathology, Wayne State Univ, Detroit, MI, USA; [5]Dept of Obstetrics & Gynecology, Semmelweis Univ, Budapest, Hungary.*

Objective: A prominent galectin, Gal-1, is expressed by the placenta and fetal membranes, is detectable in the maternal circulation, and has pleiotropic effects. This study determined if preterm labor with intact membranes (PTL) and preterm PROM in the presence/absence of intrauterine infection/inflammation (IAI) are associated with changes in the maternal Gal-1 serum concentrations.

Methods: This study included the following groups: 1) normal pregnancy (n=61); 2) PPROM with (n=36) and without IAI (n=30); 3) PTL who delivered preterm with (n=35) and without IAI (n=52); 4) PTL who delivered at term (n=45). Maternal serum concentrations of Gal-1 were determined with ELISA. qRT-PCR of Gal-1 mRNA expression was assessed in placenta and fetal membranes in PPROM.

Results: 1) Women with PPROM (with and without IAI) had higher Gal-1 concentrations than women with normal pregnancies [median: 2295.8 pg/ml (1272.9-4194.8); PPROM with IAI: 4097.9pg/ml (2181.1-10646.4) (p<0.001); PPROM without IAI: 3779.9pg/ml (1488-63549.9) (p<0.001)]; 2) Women with PTL leading to preterm delivery (with and without IAI) had higher Gal-1 concentrations than women with normal pregnancies [PTL with IAI: 4010.8 pg/ml (1002.5-205197) (p<0.001); PTL without IAI: 3725.4pg/ml (793.3-72821.5) (p<0.001); 3) Patients with PTL who delivered at term had higher Gal-1 concentrations than normal pregnant women: 3479.9pg/ml (1328.7-8200) (p<0.001); 4) The presence of inflammation was not associated with a change in maternal serum Gal-1; 5) Gal-1 mRNA expression in fetal membranes was higher in cases with histologic chorioamnionitis than those without inflammation (1.53 fold, p=0.008).

Conclusion: PTL and PPROM are associated with a higher concentration of maternal serum Gal-1 than normal pregnancy. This difference could not be accounted by the presence of IAI. However, inflammation of the chorioamniotic membranes in PPROM was associated with increased Gal-1 mRNA expression.

THURSDAY

121

The Association between Body Mass Index and Gestational Diabetes Varies by Race/Ethnicity. Amy Shah,[1] Aaron Caughey,[1] Naomi Stotland,[1] Yvonne Cheng,[1] Gladys A Ramos.[1] (SPON: Linda C Giudice). *[1]Obstetrics & Gynecology, University of California, San Francisco, San Francisco, CA, USA; [2]Obstetrics & Gynecology, University of California, San Francisco, San Francisco, CA, USA; [3]Obstetrics & Gynecology, University of California, San Francisco, San Francisco, CA, USA; [4]Obstetrics & Gynecology, University of California, San Francisco, San Francisco, CA, USA; [5]Obstetrics & Gynecology, University of California, San Francisco, San Francisco, CA, USA.*

OBJECTIVE: To examine BMI thresholds as a screening tool for gestational diabetes and whether the sensitivity varies among racial/ethnic groups.

STUDY DESIGN: This is a retrospective cohort study of pregnant women that have delivered at a single academic institution who underwent screening for gestational diabetes (GDM). Body mass index (BMI) was examined as a screening tool for gestational diabetes overall and stratified by racial/ethnic groups. Sensitivity and specificity of BMI were examined using the chi-square test and receiver-operator characteristic (ROC) curves.

RESULTS: A BMI of ≥20 kg/m2 as a screening threshold identified more than 90% of Caucasian, African-American, and Latinas with GDM, but only 80% of Asians ($p<0.001$). The sensitivity of BMI for GDM decreased steeply for Asians, but less so for Caucasians and Latinas, and least of all for African-Americans (Table).

CONCLUSION: While increasing BMI was associated with higher rates of GDM in all racial/ethnic groups, its screening characteristics varied by race/ethnicity. If only women who were overweight (BMI>25 kg/m2) were screened, 77% of African-Americans with GDM would be identified, but such a threshold would only identify 25% of Asians with GDM. While these data can be used to counsel women regarding their risk of developing GDM, BMI alone does not appear to be a particularly good screening tool.

Sensitivity of BMI for presence of GDM by ethnic groups.

	BMI >21	BMI >23	BMI >25	BMI >27
Caucasian	79.8%	60.1%	46.2%	31.3%
African American	91.5%	82.9%	76.8%	52.4%
Latina	90.1%	75.2%	58.9%	41.8%
Asian	68.4%	43.5%	24.9%	11.7%
p-value	<0.001	<0.001	<0.001	<0.001

122

Racial Disparity in VBAC Selection, Success and Complications. Alison G Cahill, David M Stamilio, Anthony O Odibo, Jeffery Peipert, Erika Stevens, George A Macones.* *Obstetrics & Gynecology, Washington University in St. Louis, St. Louis, MO, USA.*

Objective: To determine if race has an impact on election to attempt vaginal birth after cesarean (VBAC), VBAC success, and maternal morbidities associated with VBAC.

Methods: A retrospective, multi-center cohort study was conducted of women with a history of at least one prior cesarean delivery. Data were obtained on maternal demographics, medical history, antepartum and intrapartum course, delivery mode, and maternal outcomes. This analysis examines the association between race and the choice to VBAC, VBAC success rates, and maternal morbidity, including uterine rupture, and a composite morbidity outcome (uterine rupture, bladder and bowel injury, and artery laceration). Race was determined by patient self-report. Bivariate and multivariable analyses were performed to assess the independent effect of race on clinical outcomes.

Results: The cohort includes 25,076 patients with at least one prior cesarean delivery. In unadjusted and multivariable analysis, black patients were more likely to undertake a trial of labor than non-black patients, and slightly more likely to experience a failure of VBAC attempt. However, despite their increased rate of failure, black women who attempt VBAC are less likely to sustain a uterine rupture than non-black women (Table 1).

Conclusions: There is an increased rate of VBAC attempt and VBAC failure among black patients, which may reflect a disparity in counseling practices or VBAC candidate selection that should be considered. However, the increased VBAC failure rate is not associated with an increased risk of uterine rupture or over-all maternal morbidity.

A Comparison of Rate of VBAC and Associated Morbidities in Black and non-Black Patients

	Black (n=6561)	Non-Black (n=18515)	Unadjusted RR (95%CI)	Adjusted OR (95%CI)	p
VBAC Attempt	62.2%	52.2%	1.19 (1.16-1.22)	1.11 (1.03-1.19)	0.004
Failed VBAC Attempt	26.4%	23.8%	1.11 (1.04-1.18)	1.56 (1.41-1.74)	< 0.001
Uterine Rupture	0.38%	0.58%	0.65 (0.42-1.01)	0.58 (0.35-0.97)	0.037
Composite	1.66%	1.70%	0.97 (0.79-1.22)	0.89 (0.67-1.17)	0.407

122.1

A Program for Opiate Dependence during Pregnancy in a Rural Setting Improves Prenatal Care. Marjorie Meyer,*[1] Anne Johnston,[2] Dawn Plante,[1] Diantha Howard,[1] Anna Benvenuto.[1] *[1]Obstetrics Gynecology, University of Vermont, Burlington, VT, USA; [2]Pediatrics, University of Vermont, Burlington, VT, USA.*

Background: Illicit opiate use, once considered an urban problem, has increased rapidly in rural areas of the country. Current studies of the effect of opiate agonist therapy during pregnancy have been limited to urban populations. The goal of this study was to determine the impact of a program for opiate dependence during pregnancy on prenatal care (primary outcome: gestational age at start of prenatal care and gestational age of initiation of treatment for opiate dependence) and outcome (gestational age at delivery and birthweight) in a non-urban setting.

Methods: Data from 105 patients treated for opiate dependence from 2000-2005 at a single tertiary care institution in a rural setting with a wide referral base were reviewed retrospectively. From 2000-2002, women with opiate dependence were referred to the tertiary center and treated on a case by case basis. In 2002, a structured program for the treatment of opiate dependence during pregnancy was initiated in conjunction with a regional treatment center. Trend for change over time was determined for 4 groups (Kendalls tau): Group 1 (2000-2002; n=12); Group 2 (2003; n=17), Group 3 (2004; n=30) and Group 4 (2005; n=46). Data shown are median (25, 75%) or mean $\pm$std. Proportions were compared using chi-square; $p<0.05$ significant.

Results: Data are shown in Table 1. From 2000 to 2005 there was a significant decrease in both the gestational age at the start of prenatal care and the median gestational age at which opiate treatment was initiated. There was a close trend toward increased birthweight of term infants.

Conclusions: Prenatal care can be improved with treatment for opiate dependence in a rural population. Comprehensive treatment programs for opiate dependence during pregnancy should not be limited to urban settings.

Pregnancy Outcome

	2000-2002	2003	2004	2005	r	p
Gest age start prenatal care (wks; n=97)	17.2 (9.6, 23.8)	13 (10.7, 15)	11 (8, 18.7)	10 (8, 15)	-0.17	0.03
Gest age start opiate agonist therapy (wks; n=102)	19.9 (15, 27)	19 (13, 31.7)	16 (0, 22.5)	0 (0, 18.3)	-0.33	0.0001
Birthweight >37 weeks (n=85)	2946+376	2980+381	3087+537	3219+553	0.16	0.06
% preterm birth (<37 wks)(n)	8.3 (1)	23.5 (4)	27.7 (8)	13.0 (6)		0.40

123

Preterm Ovine Fetal Cellular Responses to Appetite Mediators Leptin and NPY. Radmila Runic,[1] Tri Nguyen,[1] Yousheng Jia,[2] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]UCI, Irvine, CA, USA.*

Objective: In utero nutrient restriction may result in offspring with enhanced appetite and the development of adult obesity. For appetite setpoints to be programmed in utero, central orexigenic nuclei must be responsive to appetite mediators prior to term. We sought to determine if the preterm ovine fetus demonstrates cellular (Fos) responses in the periventricular (PVN) and supraoptic (SON) nuclei following central injection of leptin and NPY.

Methods: Preterm fetal sheep (127 d; n=6) were chronically prepared with cannulas in the lateral ventricle. At 133 d, fetuses received central injection of either artificial CSF (aCSF), leptin (0.075mg/kg; Calbiochem) or NPY (0.05mg/kg; Sigma) in 1 ml. At 90 min, fetuses were sacrificed, brains perfusion fixed, and later sectioned at 35µm and immunostained for Fos (1;10,000, Calbiochem). Vectastain ABC kit (Vector Laboratories) was used for the secondary antibody-peroxidase reaction and DAB was used for staining. The supraoptic nucleus (SON) and periventricular nucleus (PVN) were examined and scored for Fos expression in their neurons. Olympus CX 31 microscope 4x magnification was used and pictures taken with Nikon E 5400 camera.

Results: Both leptin and NPY induced Fos expression in the PVN. The mean number of PVN nuclei stained for Fos was higher following both leptin (664+103) ($p<0.05$) and NPY (412+42) ($p>0.05$) as compared to control aCSF injection (190+25). SON did not demonstrate any change in Fos expression with injection of leptin or NPY.

Conclusions: The stimulation of preterm fetal Fos expression in the PVN in response to both leptin and NPY indicates that putative appetite pathways (e.g., arcuate nucleus to PVN) are responsive and likely functional in utero. These results support the potential for in utero nutrient- or stress-mediated alterations in central appetite development to program offspring food intake.

124

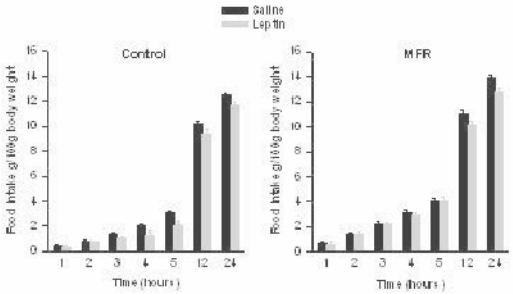
Resistance to Anorexogenic Agent Leptin in Intrauterine Growth Restricted Offspring. Mina Desai, Radmila Runic, Tri Nguyen, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective**:** Maternal food restriction (MFR) during pregnancy results in IUGR newborns which develop adult obesity, increased percent body fat, and reduced leptin-mediated satiety responses at 6 months of age. As both diet-induced and programmed (MFR) obesity are characterized by increased plasma leptin, it is unknown whether MFR offspring exhibit leptin resistance as a primary programmed or secondary obesity response. We sought to determine if young MFR offspring were resistant to the anorexic effects of leptin.

Methods**:** Control dams received ad libitum food (n=6), whereas study dams were 50% food-restricted (MFR, n=6) from pregnancy day 10 to 21. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by Control dams and were weaned at 3 wks to ad libitum feed. At 6 wks of age, anorexic effect of leptin was studied in males and females from both groups. Offspring were randomized to receive either peripheral leptin (300 µg/kg BW) or saline, and food intake was monitored for 24 h.

Results: MFR offspring at 6 wks were heavier (male: 216±7 vs 190±6 g, $p<0.01$; female: 188±4 vs 160±3 g, $p<0.01$) as compared to controls. Whereas control offspring demonstrate leptin-induced anorexigenic effects at 4 and 5 hours (Fig), 6 wk old male and female MFR offspring demonstrate an absent response to leptin.

Conslusion: MFR offspring demonstrate resistance to anorexic effects of leptin as early as 6 wks of age. Programmed impaired satiety responses contribute to increased food intake, rapid newborn weight gain, and development of adult obesity.



125

Transcriptional Regulation of Adipogenesis in Intrauterine Growth Restricted Offspring. Mina Desai,[1] Robert Lane,[2] Guang Han,[1] Michael G Ross.*[1] *[1]Obstetrics & Gynecology, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Pediatrics, University of Utah, Salt Lake, UT, USA.*

OBJECTIVE: PPARγ2 is an adipogenic transcription factor which promotes adipocyte differentiation and lipid storage. Maternal food restriction (MFR) during pregnancy results in programmed obesity in IUGR offspring. At 1 day of age, IUGR offspring exhibit increased adipose tissue PPARγ2 expression, which persists in adults. We sought to examine upstream transcriptional factors regulating PPARγ2 and its downstream targets. The C/EBP family members (C/EBPβ, C/EBPδ, and C/EBPα) activate PPARγ which then heterodimers with the retinoid X receptors (RXRα) to influence adipocyte differentiation. PPARγ2 induces SREBP1, a transcription factor which promotes lipogenesis in the adipose tissue. We determined the mRNA and protein expression of transcriptional factors regulating adipocyte differentiation in 1 day and 9 month old Control and MFR offspring.

STUDY DESIGN: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21. 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. At 1d and 9 months of age, adipose tissue was analyzed for C/EBPβ, C/EBPδ, C/EBPα, RXRα and SREBP1 for mRNA (real time RT-PCR) and protein levels (Western Blot).

RESULTS: At 1d of age, MFR pups showed significantly increased protein and mRNA expression of all C/EBP transcription factors (mRNA: C/EBPβ, 3.5-fold; C/EBPδ, 4.5-fold; C/EBPα, 5-fold; $p<0.01$), and RXRα (6.5-fold), whereas SREBP1 showed no change as compared to controls. At 9 months of age, MFR offspring continued to express persistently increased transcription factor protein and mRNA, with a further increase in C/EBPβ (6-fold) and RXRα mRNA (17-fold), continued increases in C/EBPδ and C/EBPα, and the new expression of increased SREBP1 (4-fold; $p<0.01$).

CONCLUSIONS: Though generally observed only with the development of obesity, growth restricted MFR newborns demonstrate upregulation of adipogenic transcription factors, and exhibit a further increase at 9 months of age. The increased expression of transcriptional factors upstream and downstream of PPARγ2 suggests a programmed pathway of increased adipocyte differentiation and lipogenesis which promotes the development of obesity.

126

Appetite Regulation in IUGR: Expression of Hypothalamic Insulin Receptor Substrate. Ederlen Casillas,[1] Darran N Tosh,[2] Mina Desai,[1] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Adelaide, Australia.*

Objective: Insulin acts via its receptor (IR) in the hypothalamus to inhibit feeding. Of the four known isoforms of IR substrate (IRS), only IRS-2 is implicated in neuronal control of energy homeostasis; lack of IRS-2 results in hyperphagia and obesity. Additionally, both IRS-1 and IRS-2 participate in the mediation of insulin which regulates embryonic development, postnatal somatic growth, and glucose homeostasis. Low levels of IRS-1 and IRS-2 leads to the development of insulin resistance. We have previously shown that maternal food restriction (MFR) during pregnancy results in IUGR newborns which exhibit hyperphagia and reduced anorexigenic responses. MFR adult offspring demonstrated obesity and insulin resistance as adults. We sought to determine if altered hypothalamic IRS-1 and IRS-2 contribute to reduced satiety responses in IUGR offspring.

Method**:** Pregnant control dams received ad libitum (n=5) food, whereas study dams were 50% MFR (n=5) from pregnancy day 10 to 21 to produce IUGR newborns. At postnatal day 1, brains were collected. The hypothalamic region was dissected and analyzed for mRNA levels of IRS-1 and IRS-2 by real time RT-PCR. Data is presented as fold difference normalized to 18sRNA. Values shown are mean ± SE.

Results**:** At 1d of age, IUGR pups had lower body weights (6.2±0.3 vs 7.6±0.3 g, $p<0.01$) and significantly reduced blood glucose (94±3 vs 102± 4 mg/dl, $p<0.01$). IUGR pups showed significantly decreased mRNA expression of IRS-1 (0.2-fold) but not IRS-2 as compared to control pups.

Conclusion**:** If replicated in peripheral tissues, the downregulation of IRS-1 is consistent with reduced body weight and plasma glucose levels. The unaltered IRS-2 at day 1 suggests that enhanced appetite and reduced satiety responses of IUGR offspring may be due to programmed anorexigenic pathways other than insulin-mediated. In view of reduced anorexigenic responses to leptin and low plasma glucose, we propose that altered adipose-hypothalamic signaling contributes to offspring obesity.

127

Perifollicular Undernutrition Increases Fetal Growth and Development during Early Gestation in the Sheep. Severence M MacLaughlin,[1] Simon K Walker,[2] IC McMillen.[1] (SPON: David M Olson). *[1]Sansom Institute, Adelaide, South Australia, Australia; [2]South Australian Research and Devleopment Institute, Rosedale, South Australia, Australia.*

Recent studies have shown that maternal undernutrition imposed during a 1-2 month period before and in the first week after conception can alter embryonic, placental and fetal growth. It is unclear to what extent the effects of undernutrition during the period of follicular and oocyte development (PFUN), are responsible for this altered fetoplacental development.

Objective:

We have therefore tested the hypotheses that maternal undernutrition imposed only during the period of follicular and oocyte development can alter the fetoplacental growth trajectory during early gestation and the perturb the relationship between maternal weight at conception and fetoplacental growth during early gestation.

Methods:

Ewes were maintained on either a control (100% maintenance, n = 10) or a PFUN diet (70% of control feed allowance from at least 52 days, restored to 100% for 13.3 ± 1.5 days before conception, n=7). All ewes were fed a maintenance diet of 100% of nutritional requirements from mating to ~55 d gestation. Fetal and placental weights were measured at postmortem in all pregnancies at ~55 d gestation.

Results:

Fetal weight ($P < 0.01$) and crown rump length ($P < 0.01$) was significantly increased in the PFUN compared to control group. The feto-placental weight ratio was maintained in the PFUN group and there was an increase ($P<0.02$) in the number of placentomes per pregnancy in PFUN compared to control pregnancies. In control, but not PFUN, pregnancies there was a positive

relationship between maternal weight at conception and placental weight at ~55 d gestation. Similarly there were significant relationships between mean placentome size and maternal weight at conception in control, but not PFUN pregnancies.

Conclusions:

This study is the first to demonstrate that maternal nutrition during the period of follicular and oocyte development, alone, can alter the fetoplacental growth trajectory. In addition, exposure of a developing oocyte to maternal nutrition during follicular development ablates the relationship between maternal weight at conception and uteroplacental growth at ~55 d gestation. Thus, PFUN alters fetal growth through placental alterations. PFUN may impact on the maternal mRNA pool, responsible for embryo development up to the maternal zygotic transition, to alter the development of cells predestined to differentiate into trophectoderm tissue.

128

Effects of Chronic Prenatal Stress on Adult Body Weight, Body Fat and Leptin Concentrations in Male Rats. Lisa A Baer,[1,2] Charles E Wade,[2] April E Ronca.[*3] *[1]US Army Institute of Surgical Research, Fort Sam Houston, TX, USA; [2]Life Sciences Division, NASA Ames Research Center, Moffett Field, CA, USA; [3]Obstetrics & Gynecology and Neurobiology & Anatomy, Wake Forest School of Medicine, Winston Salem, NC, USA.*

Objectives: We tested the 'fetal programming' hypothesis that male rat pups conceived, gestated and born during exposure to chronic stress induced by continuous 20 rpm centrifugation are characterized by low birth weight and adult overweight, along with an increase in total body fat and leptin. **Methods:** Young adult male and female rats were adapted to centrifugation for one week prior to mating, conception and gestation of prenatal offspring. Centrifugation was discontinued at birth and centrifuged (CF) and non-centrifuged (nCF) control neonates were fostered to non-manipulated, newly parturient dams. **Results:** As compared nCF pups, birth weights of CF pups were significantly lower (Mean ± s.e.: nCF, 7.49 ± 0.22gm, CF, 6.30 ± 0.11gm; $p<0.01$). Their body weights remained significantly ($p<0.05$) lower until Postnatal day (P)12. On P90, body weights of CF males were significantly greater those of nCF males (Mean ± s.e.: nCF, 487 ± 7 gm; CF, 519 ± 11 gm; $p<0.02$). Using Total Body Electrical Conductivity (TOBEC) to determine live body composition, total body fat mass was significantly higher in CF than nCF males (Mean % ± s.e.: nCF, 44±0.32; CF, 45 ± 0.47; $p<0.02$). The appetite hormone, leptin, was also significantly ($p<0.05$) elevated in CF relative to nCF males. **Conclusions:** Taken together, our data suggest that chronic prenatal 'stress' alters the intrauterine milieu, thereby 'programming' persistent adult overweight with correlated changes in body fat mass and leptin.

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129

***In Utero* Nicotine Exposure and Postnatal Diet: Implications for Future Health.** Sarah D McDonald,[1] Maria A Querques,[1] Katherine M Morrison,[2] Alison C Holloway.[*1] *[1]Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada; [2]Pediatrics, McMaster University, Hamilton, ON, Canada.*

Introduction: Recent epidemiological studies have shown an increased risk of obesity in children born to women who smoked during pregnancy. It is unclear whether this obesity is a result of permanent alterations in the metabolism of the offspring, postnatal diet, a combination of these or other confounding factors. An animal model of tobacco exposure has been developed to address the issue, and has shown that nicotine alone at concentrations that are representative of those found in women who smoke, causes postnatal obesity in male rats. However, the contribution of postnatal diet has not yet been examined.

Objective: In this study, we examined the interaction between fetal and neonatal exposure to nicotine and postnatal diet on the development of obesity and dysglycemia in male offspring.

Methods: Female nulliparous Wistar rats (N= 10 per group) were given saline (vehicle) or nicotine (1mg/kg/d) ("treatment") daily via subcutaneous injection for 2 weeks prior to mating until weaning. At weaning pups were randomly assigned to receive either a low fat diet ("LF", 5% fat, 3.82 Kcal/g,) or a hypercaloric high fat diet ("HF", 21% fat, 4.7 Kcal/g). Weekly from postnatal day 1, the pups were weighed and food consumption was calculated. At week 7, the pups underwent a glucose tolerance test (2g/kg). The animals were sacrificed and fat pad weight was determined.

Results: Saline treated pups on the HF diet were dysglycemic compared to those on the LF diet. Interestingly, nicotine exposed pups raised on the HF diet did not develop dysglycemia. Compared to saline treated pups, nicotine exposed pups on the LF diet had higher serum triglyceride concentrations, however there were no significant differences in pups receiving the HF diet. Irrespective of treatment, pups on the HF diet had significantly increased fat pad weight, although there was no difference in caloric intake. There was no effect of diet or treatment on body weight, fasting glucose or insulin.

Conclusion: Compared to a low fat postnatal diet, a HF diet results in dysglycemia in pups exposed to saline, but not nicotine *in utero* and during lactation. Exposure to nicotine does not result in obesity at 7 weeks of age, but does result in increased triglycerides in pups on a LF diet.

Acknowledgement: This research was supported by the Molly Towell Perinatal Foundation.

130

High-Protein Diet Improves the Fetal Growth Restriction by Maternal Food Restriction, Partly by Augmentation of the Gene Expressions of Insulin-Like Growth Factors (IGFs) in Fetal Liver. Haruta Mogami,[1] Hiroaki Itoh,[*1] Shigeo Yura,[1] Makoto Kawamura,[1] Norimasa Sagawa,[2] Shingo Fujii.[1] *[1]Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto City, Kyoto prefecture, Japan; [2]Department of Obstetrics and Gynecology, Mie University Graduate School of Medicine, Tsu City, Mie Prefecture, Japan.*

Objectives: Maternal undernutrition causes fetal growth restriction (FGR). It has been experimentally shown that low-protein diet also causes FGR in pregnant animals. It implies that sufficient maternal protein intake is necessary for appropriate fetal growth. On the other hand, insulin-like growth factors (IGFs) play a key role in the regulation of fetal growth. Using a mouse model of undernutrition during pregnancy, we investigated whether maternal food protein increment affected the fetal growth and the change of the IGFs gene expressions in placenta and fetal liver.

Methods: Pregnant C57Bl/6 mice were food-restricted to 70% (undernutrition, UN) of the control group (normal nutrition, NN) from 10.5 days post-coitum (dpc) to 18.5 dpc using normal protein diet (20% protein included). In another group (undernutrition with high-protein, P-UN), food restriction to the same calorie intake as UN group were performed using high-protein diet (40% protein included). Fetuses and placentas were collected on 18.5 dpc, and their weight was measured. The gene expressions of IGF-I, -II, and type 1 IGF receptor in fetal liver and placenta were measured by quantitative RT-PCR. All experimental procedure was approved by the institutional Animal Research Committee.

Results: The fetal weight of UN (0.88±0.01g) was significantly smaller than that of NN (1.05±0.01g), whereas that of P-UN (0.93±0.01g) was significantly larger than those of UN, regardless of the same extent of maternal calorie restriction. In fetal liver, IGF-I and -II mRNA expressions of P-UN were significantly increased compared to that of UN (+54% and +33% respectively). In placenta, IGF-I mRNA expression of P-UN was significantly higher than of UN (+76%). IGF-II mRNA levels showed no significant difference among these groups in placenta. The gene expression of type 1 IGF receptor was not changed among NN, UN, and P-UN in both fetal liver and placenta.

Conclusions: The food protein increment in undernourished pregnant mice improved fetal growth restriction. This improvement may be attributed at least partly to the elevated gene expression of IGF-I and -II in the fetal liver.

131

Impact of Increased Maternal Nutrition on the Expression of Genes Regulating Gluconeogenesis in the Liver of the Postnatal Lamb. L Rattanatray,[1,2] BS Muhlhausler,[1] IC McMillen.[2] (SPON: David M Olson). *[1]Early Origins of Adult Health Research Group, Sansom Institute, University of South Australia, Australia; [2]School of Molecular and Biomedical Science, The University of Adelaide, Australia.*

Objective: Epidemiological studies have demonstrated a clear positive association between exposure to excessive nutrition before birth and the incidence of the metabolic syndrome and type 2 diabetes in later life. We have previously demonstrated that lambs of overnourished ewes are hyperglycemic but it is unknown whether exposure to overnutrition in late gestation alters the expression of hepatic gluconeogenic enzymes in early postnatal life.

Hypothesis: We have investigated the hypothesis that increased prenatal nutrition increases hepatic glucose output by upregulating hepatic gluconeogenic capacity.

Methods: Ewes were provided with either 100% (Control, C, n=8) or 160% (Well fed, WF, n=8) of maintenance energy requirements (MER) from 115 d gestation until delivery. Postmortem was performed on postnatal day 30. The relative hepatic expression of key regulatory (PGC-1, PPARα) and gluconeogenic genes (PEPCK, G6PHOS) were determined by qRT-PCR.

Results: Relative liver weight was significantly higher in lambs of WF ewes compared to Controls (21.7 ± 0.591 g/kg vs19.4 ± 0.57 g/kg, P<0.05) and was directly related to maternal ME intake (%MER) between 125-134d gestation when data from all lambs were combined (r^2=0.31; P=0.008). There were no differences in expression of G6PHOS, PGC-1 and PPARα between Control and WF groups. The expression of PPARα (r^2=0.43; P=0.02), PEPCK r^2=0.20; P<0.05) and G6PHOS (r^2=0.35; P<0.01) was, however, directly related to expression of PGC-1. There was a trend towards decreased expression of PEPCK in lambs of WF ewes (0.16 ± 0.05 vs. 0.39 ± 0.03, P=0.06), and PEPCK expression was inversely correlated with total relative fat mass (r^2=-0.25; P=0.025) when data from all lambs were combined.

Conclusions: We have therefore demonstrated that exposure to increased maternal nutrition before birth promotes liver growth in early postnatal life, but is not associated with an increase in hepatic PEPCK expression. These data suggest that PEPCK expression may be suppressed by hyperglycemia in lambs of well fed ewes in early postnatal life. It is also possible, however, that the increase in relative liver weight in lambs of well fed ewes may potentially contribute to an increase in overall gluconeogenic capacity, and therefore postnatal hyperglycemia, in these animals.

132

Baboon Fetal Liver IGF System mRNA and Protein Changes in Response to Maternal 70% Global Reduction in Food Consumption. Cun Li,[1] Natalia E Schlabritz-Loutsevitch,[1] Victor KM Han,[2] Karen Nygaard,[2] Peter W Nathanielsz,[*1] Thomas J McDonald.[*1] *[1]Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, TX, USA; [2]Paediatrics and Child Health Recearch Institute, University of Western Ontario, London, ON, Canada.*

Introduction: Information on nutritional effects on placental and fetal growth and development is critical to understanding pregnancy physiology and pathophysiology. While responses of maternal and fetal IGF systems to controlled maternal nutrient restriction (MNR) during pregnancy have been studied in rodents and sheep, few data exist in nonhuman primates. We determined effects of MNR in baboons on the fetal hepatic IGF system.

Methods: Baboons, fed *ad libitum (*CTR, n = 8) or 70% of CTR diet (MNR, n = 6) from 0.16 - 0.5 gestation (G), were C-sectioned at 0.5G with fetuses necropsied. The following were measured in the right liver lobe: IGF-2 and IGFBP-3 mRNA by *in situ* hybridization (ISH), IGF-II, IGF-R1, IGF-R2, IGFBP-1 and IGFBP-3 protein by immunohistochemistry (IHC) and glycogen concentrations by the periodic acid Schiff reaction (PAS). ISH and IHC were quantified by image analysis which calculated fraction (area hybridized or immunostained ÷ region of interest x 100%). PAS was quantified by measuring staining intensity. Statistical comparisons were by Rank sum test. Data are expressed as mean ± SEM with CTR presented first.

Results: Fetal: body wt. (733.3 ± 39.8 vs. 607.0 ± 90.1 g, n.s.) and liver wt. (20.0 ± 0.7 vs. 16.4 ± 2.8 g, n.s.). Serum: IGF-I (365.12 ± 106. 3 vs. 17.1 ± 18.9 ng/ml, *p* = 0.06), growth hormone (3.0 ± 0.99 vs. 1.0 ± 0.32 ng/ml n.s.), IGFBP-3 (0.57 ± 0.03 vs. 3.47 ± 2.11) and IGF-I:IGFBP-3 ratio (4.85 ± 1.7 vs. 1.4 ± 1.3, p< 0.05). Liver glycogen (197.5 ± 11.7 vs. 162.8 ± 11.7 pixels, p< 0.05). See Table 1 for more results.

Conclusions: Despite the lack of effect on fetal body or liver weight, marked changes in several key components of the IGF system suggest compensatory changes in response to MNR and confirm that body weight is a poor marker for intrauterine growth restriction effects.

Table 1. mRNA and protein for IGF system members in liver of CTR and MNR fetuses at 0.5G.

	mRNA IGF-2	mRNA IGF-BP1	Protein IGF-2	Protein IGF-1R	Protein IGF-2R	Protein IGF-BP1	Protein IGF-BP3
CTR	16.3 ± 2.4	2.5 ± 0.4	15.0 ± 4.5	1.9 ± 0.3	45.7 ± 8.0	9.8 ± 2.3	2.8 ± 0.7
MNR	5.2± 1.6*	5.5 ± 1.3**	2.6 ± 0.9*	0.2 ± 0.1*	14.9 ± 6.1*	62.6 ± 9.8*	27.2 ± 4.5*

Different from CTR *p< 0.05, ** p=0.06

133

Effects of 30% Maternal (M) Nutrient Restriction (NR) on mRNA and Protein Expression of Selected IGF System Members in Baboon Placenta at 90 Days Gestation (dG). Cun Li,[1] Natalia E Schlabritz-Loutsevitch,[1] Victor KM Han,[2] Karen Nygaard,[2] Mark J Nijland,[1] Thomas J McDonald,[*1] Peter W Nathanielsz.[*1] *[1]Obsterics and Gynecology, University of Texas Health Science Center, San Antonio, TX, USA; [2]Paediatrics and Child Health Research Institute, University of Western Ontario, London, ON, Canada.*

Introduction: IGF system members influence fetal growth: 1) indirectly by effects on placental growth thereby modulating the maternal-fetal interface and 2) directly by effects on the fetus. While effects of NR on serum and tissue concentrations of IGF system members have been studied in a number of species [Placenta 24:803], to our knowledge few data are available about NR effects on mRNA and protein expression of IGF system members other than IGF-I in a nonhuman primate. We examined MNR effects on placental IGF-II, IGF- receptor (R)1, IGF-R2, IGF binding protein (BP)-1 and IGFBP-3 mRNA and protein expression at 50% of gestation in the baboon. We have previously reported that MNR reduces IGF-I in 90 dG placentas -Term 180dG [SGI 53rd Ann. Mtg. Abst. 157].

Methods: From 30 to 90 dG, female baboons were fed *ad lib* (CTR; n = 8) or fed 70% CTR diet (MNR; n = 6) with necropsy and tissue collection at 90 dG. mRNA expression was measured by *in situ* hybridization histochemistry and for protein by immunohistochemistry. For both analyses, quantification by image analysis calculated fraction (area hybridized or immunostained ÷ region of interest x 100%) and statistical comparisons were made by rank sum test. Data are expressed as mean ± SEM with CTR presented first.

Results: Placental data are: weight (70.4 ± 5.1 vs. 62.9 ± 1.5 g), volume (30.20 ± 6.3 vs. 14.8 ± 2.9 ml, p = 0.05) and those in Table 1.

Conclusion: In contrast to the present study, 30% MNR in the guinea pig results in decreased IGF-II [J. Endocrinology 179:437]. When combined with our previous finding of decreased placental IGF-I in this model of MNR, the increase in IGF-II protein found herein may be a consequence of the decrease in IGF-R2 since this receptor functions primarily in the degradation of IGF-2.

Table 1. mRNA and protein for IGF system members in placenta of CTR and MNR fetuses at 0.5G.

	mRNA IGF-2	mRNA IGF-BP1	mRNA IGF-BP3	Protein IGF-2	Protein IGF-1R	Protein IGF-2R	Protein IGF-BP3
CTR	1.5 ± 0.5	4.8 ± 1.1	2.1 ± 0.6	7.2 ± 1.6	15.2 ± 3.9	9.0 ± 3.0	20.3 ± 3.8
MNR	8.7 ± 4.2**	4.9 ± 1.3	7.6 ± 3.1**	22.6 ± 1.3*	52.8 ± 5.9*	1.2 ± 0.3*	13.0 ± 3.6

Different from CTR *p< 0.05, ** p = 0.06.

134

Direct Effect of Melatonin on Sheep Fetal Brown Adipose Tissue (BAT). Francisco J Valenzuela,[1] Mauricio Mondaca,[1] Claudia Torres-Farfan,[1] Guillermo J Valenzuela,[*2] Raquel Riquelme,[3] Eemilio A Herrera,[1,4] Anibal J Llanos,[1,4,5] Maria Seron-Ferre.[1,5] *[1]ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile; [2]Women's Health, Arrowhead Regional Medical Center, Colton, CA, USA; [3]Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Chile; [4]INCAS, Universidad de Chile, Chile; [5]Universidad de Tarapaca & CIHDE, Arica, Chile.*

Fetal BAT, accrued during pregnancy, is important for newborn thermogenesis. In the fetus, BAT thermogenic function is inhibited by factors produced or transported by the placenta. Maternal melatonin is transferred across the placenta and melatonin receptors are present in several fetal tissues. To asses whether maternal melatonin may be involved in regulation of fetal BAT function we investigated: a) the presence of a melatonin receptor in fetal BAT and b) whether this receptor is functional by measuring *in vitro* the effect of melatonin on BAT glycerol production stimulated by norepinephrine (NE).

Material and Methods: Perirenal BAT and pars tuberalis were collected from three sheep fetuses (90% gestation). A piece of BAT was frozen for membrane preparation and another was preserved in TRIzol for RT-PCR. The presence of a melatonin receptor was investigated by ^{125} I-iodomelatonin binding to membranes and the isoform of the receptor expressed was identified by RT-PCR, product isolation and sequencing. Fetal pars tuberalis was used as positive control. The remaining BAT was cut in explants (~25 mg) and incubated during 6 hours with 0.01 to 10 mM of NE and 0,1 and 10 nM melatonin. Supernatants were collected and the production of glycerol was measured by the glycerol oxidase method.

Results. Fetal BAT membranes show the presence of a melatonin receptor (Kd: 44.2±12 pM and Bmax: 2.0±0.2 fmol/mg protein; n=3). By RT-PCR we found that sheep fetal BAT expressed a MT2 melatonin receptor. Pars tuberalis (positive control) expressed only the MT1 melatonin receptor as previously known

In culture, fetal BAT explants increased glycerol production in response to 1 and 10 uM NE. This increase was abolished by addition of 1 and 10 nM Melatonin to the culture, consistent with the presence of a functional melatonin receptor.

Conclusions. The expression of a MT2 receptor and the evidence of direct melatonin actions suggest that Melatonin may be involved in regulation of fetal BAT function.

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THURSDAY

135

The Observed Hyperglycemia, but Not the Hypoinsulinemia, in Response to a Glucose Load in Male Growth-Restricted Offspring Can Be Ameliorated by Improved Lactational Nutrition. Andrew L Siebel,[1] Kerryn T Westcott,[1] Amy Mibus,[1] Miles J De Blasio,[2] Julie A Owens,[2] Mary E Wlodek.*[1] *[1]Physiology, Univ of Melbourne, Parkville, VIC, Australia; [2]Obstetrics & Gynaecology, Univ of Adelaide, Adelaide, SA, Australia.*

Objectives: Intrauterine growth restriction and its associated accelerated postnatal growth increase the risk of developing adult diseases, including diabetes. Placental restriction impairs both pre- and postnatal nutrition and programs insulin resistance in the adult rat. We have used cross-fostering to assess the influence of prenatal and postnatal restraint on insulin secretion and glucose response.

Methods: Bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) was performed on day 18 of gestation in Wistar Kyoto rats. Control, Reduced (RED; reducing litter size of controls on day 1 to match R) and Restricted pups were cross-fostered onto Control or Restricted mothers 1 day after birth. At 6 months of age, a catheter was inserted into the carotid artery of the anaesthetised rats. After adequate equilibration, glucose (0.5g/kg) was administered intra-arterially (IAGTT). Blood was collected before and after the glucose bolus for up to two hours. Plasma insulin (LINCO RIA) and glucose (COBAS) concentrations were measured in male and female offspring in response to the IAGTT.

Results: Male, but not female, R-on-R offspring showed marked hyperglycemia ($p<0.05$), 1 minute after a glucose bolus when compared to C-on-C offspring. The plasma glucose peak concentration in male R-on-C offspring was intermediate between C-on-C and R-on-R. One minute after a glucose load, R-on-R males had lower ($p<0.05$) plasma insulin concentrations than C-on-C males, with impaired first-phase insulin secretion.

Conclusions: Being born small and then exposed to an impaired lactational environment (R-on-R) adversely effects adult glucose homeostasis and insulin secretion in males only. Correcting the poor lactational environment for pups born small (R-on-C) partially improves this hyperglycemic response to a glucose load. Understanding how the prenatal and postnatal environments influence insulin secretion and glucose homeostasis will assist in developing targeted treatments, leading to improved adult health.

136

C57BL/6J Male Offspring Exposed *In Utero* and during Weaning to a Maternal Low Protein Diet Have Reduced Muscle Weight by 12 Months of Age. Marta Fiorotto,[1] Rebecca Rosetta,[1] Zhiyin Yu,[2] William Oliver,[1] Ignatia Van den Veyver.*[2,3] *[1]Pediatrics-Nutrition; [2]Obstetrics and Gynecology; [3]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.*

Objective: C57BL/6J mice are predisposed to obesity and type 2 diabetes (T2DM) and may be a sensitive model for fetal programming of T2DM. The "maternal low protein diet" (MLP) rat model is well established to study fetal growth restriction effects on programming of T2DM. How data from C57BL/6J mice compare to rat is incompletely known. Here, we evaluate glucose tolerance and tissue weights in C57BL/6J male offspring exposed *in utero* and during suckling to MLP.

Methods: Female mice were fed diets containing 8% (MLP) or 20% protein (control) starting 4 weeks prior to mating. Litters were culled to 6 on day 3 (P3). After weaning, male offspring were single-housed, fed a standard diet and weighed weekly until 12 weeks, then monthly until 1 year. Intraperitoneal glucose tolerance tests were performed at P21, 6 months and 1 year (n=6 per diet). Mice were sacrificed at 1 year. Heart, kidneys, pancreas and liver were removed and weighed. Individual hindleg muscles were dissected and weighed. Tibial bone lengths were measured. (Statistics: t-test and ANOVA)

Results: At P365, MLP offspring were lighter than controls (27.7+/-2.5g vs. 31.2+/-1.5g, p=0.02). Weight of organs (as % of total body weight) was not significantly different between MLP and control offspring (heart: 0.69+/-0.13% vs. 0.67+/-0.16%, p=0.77; liver: 4.87+/-0.62% vs. 4.52+/-0.66%, p=0.39; pancreas: 1.01+/-0.13% vs. 0.89+/-0.09%, p=0.12; kidneys: 1.80+/-0.20% vs. 1.73+/-0.16%, 0.58). Tibiae (18.00+/-0.19mm vs. 18.37+/-0.15mm, p=0.02) were shorter in MLP offspring. Standardized muscle weights (average weight/mm tibia length) were lower for tibialis anterior (2.22+/-0.19g vs. 2.63+/-0.10g, p=0.001), gastrocnemius (6.25+/-0.67g vs. 7.18+/-0.41g, p=0.01), soleus (0.41+/-0.03g vs. 0.49+/-0.03g, p=0.005) and extensor digitorum longus (0.52+/-1.00 vs. 0.62+/-0.04, p=0.05), but not for quadriceps (9.67+/-0.47g vs. 10.67+/-0.57g, p=0.07) and plantaris (1.02+/-0.8g vs. 0.97+/-0.07g, p=0.34) (MLP: n=5; control: n=6). All had abnormal GTT at 6 months and 1 year; there was no difference between MLP and controls.

Conclusions: C57BL/6J males exposed to MLP have lower total body weight and muscle weight at 1 year of age. The molecular change(s) underlying the reduced muscle mass will be studied for their role in programming of T2DM.

137

Vitamin C Desensitizes Baroreflex Function in Fetal Life. Avnesh S Thakor, Dino A Giussani.* *Physiology, Cambridge, United Kingdom.*

Introduction: Reactive oxygen species (ROS) have been implicated in the pathogenesis of vascular dysfunction in several circulations (Chen and Keaney. *Endothelium* **11(2)**:109,2004), including the placenta (Davidge. *Sem Rep End* **16**:65, 1998). Hence, antioxidants such as vitamin C, are being considered for treatment of pregnant women with pre-eclampsia. However, little, if any, consideration has been given to the potential effects of vitamin C in programming cardiovascular function in the developing fetus. We investigated the effects of vitamin C on baroreflex function in the late gestation fetus *in vivo*.

Methods: Under anesthesia, 5 fetal sheep (0.8 gestation) were instrumented with vascular catheters. Five days later, fetuses received bolus doses of norepinephrine (5-75µg) i.a. On separate days, this was repeated during treatment of the fetus i.v. with saline, or vit C (25 $mg.min^{-1}$) or vit C during NOS blockade. Baroreflex curves were constructed for each fetus by plotting for each dose, maximal increases in arterial blood pressure against maximal changes in heart rate or pulse interval.

Results: Arterial blood gases for all fetuses on each study day were all within normal range. All fetuses showed a reduction in baroreflex sensitivity (from 8.5±2.4 to 4.0±2.7 msec/mmHg) during vitamin C treatment ($P<0.05$), which was recovered with vitamin C treatment during NOS blockade (8.4±1.9 msec/mmHg).

Conclusion and Implications: Vitamin C reduces the sensitivity of the fetal arterial baroreflex *in utero* via a NO dependent mechanism. Clinical trials that involve maternal supplementation with vitamin C may be inadvertently reprogramming fetal baroreflex function, which, in turn, may predispose offspring to develop hypertension in later life.

Journal of Experimental Pathology and The Lister Institute for Preventive Medicine.

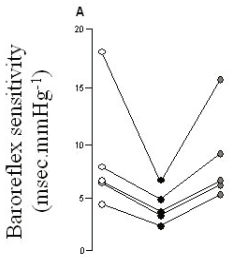
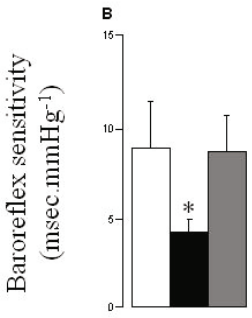


Figure 1. Cardiac baroreflex vagal sensitivity (pulse interval-blood pressure gradient) in the ovine fetus

(A) The change in cardiac baroreflex vagal sensitivity during saline infusion (○), treatment with vitamin C (8.9±0.4 $mg.kg^{-1}.min^{-1}$) alone (●) or treatment with vitamin C during NOS blockade (⊙), for five individual fetuses.



(B) Bars represent the mean±S.E.M. for cardiac baroreflex vagal sensitivity during saline infusion (□; n=5), treatment with vitamin C (8.9±0.4 $mg.kg^{-1}.min^{-1}$) alone (■; n=5) or treatment with vitamin C during NOS blockade (▩; n=5). *$P < 0.05$, *vs.* saline infusion

138

Response of Microvascular Endothelial Cells to Glucocorticoids Is Programmed *In Utero*. Omid Khorram, Guang Han, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Offspring of maternal food restricted (MFR) dams exhibit adult hypertension in association with a reduced microvascular branching pattern and altered extracellular matrix. We previously showed endothelial cells from MFR offspring have decreased expression of VEGF, and increased glucocorticoid (GC) receptors. In view of the potential for increased GC endothelial signalling, we hypothesized that GCs inhibit expression of VEGF in MFR endothelial cells.

Methods: Pregnant Sprague-Dawley rats had 50% MFR from day 10 of gestation until delivery. Offspring were sacrificed on day 1 after birth. The lungs from 6 control and 6 food restricted offspring derived from 6 different litters were dissected and the outer 3-5 mm peripheral lung tissue was sharply dissected. Microvascular endothelial cells were isolated using the method of Magee et al (AJP11:L433). This method involves digestion of tissue with 0.5% collagenase, passage serially through nylon filters of varying size, and growth of cells in L-valine-free media containing endothelial cell growth factor. Staining of cells with Von Willerbrand factor showed greater than 95% purity of cells. Cells were used between 3-5 passages. After 24 hours of exposure to Dexamethasone (10^{-4} M) alone or in combination with RU486 (10^{-5} M) cells were harvested and total RNA extracted and subjected to Real time RT-PCR using primers for rat VEGF.

Results: Dexamethasone induced a highly significant ($P<0.001$) decrease in expression of VEGF in microvascular endothelial cells derived from MFR offspring but not in cells obtained from control offspring. RU486 had no effect alone on VEGF expression in either dietary group, and completely blocked the inhibitory effect of dexamethasone on VEGF in MFR cells.

Conclusion: These results support our hypothesis that increased expression of GC receptors and therefore sensitivity to GC in endothelial cells of MFR offspring results in suppression of VEGF expression and reduced microvascular branching.

139

***In Utero* Undernutrition Induces Marked Changes in Offspring Microvascular VEGF Expression: Mechanism of Programmed Hypertension.** Omid Khorram, Naseem Khorram, Mina Desai, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Offspring of maternal food restricted (MFR) dams exhibit reduced microvascular density, decreased newborn thoracic aorta VEGF expression and adult hypertension. We sought to determine if impaired microvascular angiogenesis in MFR offspring is secondary to decreased expression of VEGF.

Methods: Pregnant Sprague-Dawley rats were food restricted by 50% as compared to control animals from e10 of gestation until term. At birth offspring were cross fostered to dams fed an ad libitum diet. Offspring were sacrificed on e21, day 1 after birth and at 2 months of life. The mesentery and thoracic aortas were dissected and fixed in 4% paraformaldehyde. Paraffin sections (5μm) of aortas and mesenteric arterioles were made. Sections were immunostained using a polyclonal antibody which recognizes rat VEGF. Digital images were then obtained and analyzed using the Image Pro Plus software in a blinded fashion. The integrated optical density (IOD) was then compared between the controls and MFR vessels.

Results: There was a marked decrease in VEGF IOD in e21 (Control 34.4 ± 2.4 vs MFR 20.0 ± 2.8, $P<0.01$) and 1 day old (Control 30 ± 1.6 vs MFR=22.8 ± 1.8, $P<0.01$) MFR mesenteric microvessels. At 2 months of life VEGF expression pattern reversed with significantly greater expression in mesenteric arterioles of MFR offspring as compared with controls (Control 2.98 ± 0.4 vs MFR=6.3 ± 0.9, $P<0.01$). VEGF expression was similar in both genders, and was predominantly within the endothelial cells and vascular smooth muscle cells.

Conclusion: These results support our hypothesis that in utero undernutrition induces an inhibition of microvascular VEGF expression and therefore reduced angiogenesis. We propose that postnatal upregulation of VEGF in MFR vessels induces local inflammation (via T_H2 cytokines) contributing to development of hypertension.

140

A Novel Mechanism for Maternal Food Restriction (MFR)-Induced Glomerular Sclerosis. John S Torday,[1,2] Mina Desai,[1] Virender K Rehan,[2] Michael G Ross.*[1] [1]*Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA;* [2]*Dept. of Pediatrics, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Adult offspring of MFR dams, exhibit obesity, hypertension and reduced glomerular number, though the cellular/molecular signaling processes for glomerular scarring is unknown. We hypothesize that MFR-induced hypertension and obesity would cause elevated circulating endothelin (ET-1) and leptin, respectively, stimulating glomerular collagen deposition, remodeling and scarring.

Methods: Rat dam food intake was reduced by 50% from e10 to term. At birth, offspring were culled to 4 males and 4 females. To examine the effects of leptin, exogenous leptin (300μg/kg body weight for 1 hr) was administered to randomly selected pups at 3 wks. MFR, leptin treated and control animals were sacrificed at 3 weeks and 8 weeks, and kidney sections analyzed by standard morphometric methods and quantified using Image Pro™ software. mRNA expression was determined using PCR; leptin and endothelin-1 levels were determined by ELISA.

Results: Despite low birth weight, MFR offspring were heavier than controls at 3 wks with associated elevated plasma leptin (1.48 ± 0.10 vs 1.01 ± 0.07 ng/ml, $p<0.01$) and circulating ET-1 (166 ± 20 vs 82 ± 17 ng/ml, $p<0.01$). Exogenous leptin treatment of 3 wk old MFR offspring caused significant increases in kidney mRNA expression of both the ObRa (1.5 ± 0.1 vs 1.0 ± 0.1 AU, $p<0.01$) and ObRb (1.6 ± 0.1 vs 1.0 ± 0.1 AU, $p<0.002$) isoforms of the leptin receptor, suggesting increased leptin signaling. The increase in leptin signaling was associated with increased collagen type I mRNA expression at 3 to 8 wks of age, consistent with the glomerular hypertrophy and scarring observed histologically.

Conclusions: These results demonstrate that exogenous leptin induced renal leptin receptor and collagen expression, and are consistent with a novel hypothesis for glomerular hypertrophy/glomerular sclerosis involving direct effects of leptin and ET-1 on glomerular collagen deposition and consequent structural changes in the glomeruli. We propose that offspring hypertension-induced ET-1, and adiposity-induced hyperleptinemia are putative mechanisms contributing to glomerular scarring and the reduced glomerular number observed in MFR offspring.

141

The Effects of Maternal Food Restriction (MFR) on Rat Offspring Kidney Structure. John S Torday,[1,2] Mina Desai,[1] Virender K Rehan,[2] Michael G Ross.*[1] [1]*Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA;* [2]*Dept. of Pediatrics, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: MFR during pregnancy produces offspring with reduced glomerular number and adult hypertension, though the mechanism of impaired renal development is unknown. We hypothesize that MFR-induces offspring glomerular hypertrophy, remodeling and scarring. We sought to characterize the development of phenotypic changes in MFR-offspring kidney structure.

Methods: Rat dam food intake was reduced by 50% from e10 to term. At birth, offspring were culled to 4 males and 4 females. Randomly selected animals were sacrificed at birth, 3 weeks and 8 weeks, and kidney sections analyzed by standard morphometric methods and quantified using Image Pro™ software. At 12 weeks of age, systolic blood pressure was determined using the tail-cuff method (ML125 NIP System, AD Instruments) in Control and MFR male offspring previously acclimatized to restraint (5 recordings of blood pressure after 30 min baseline period).

Results: MFR induced hypertension in rat offspring with systolic blood pressure significantly elevated at 12 wks of age (152±3 vs 146±2 mmHg, $p<0.05$). Glomerular surface area was significantly increased in MFR as compared to control offspring at both 3 weeks (2.0±0.3 vs 1.5±0.1 nm^2, $p<0.01$) and at 8 weeks (2.2±0.3 vs 1.5±0.2 nm^2, $p<0.01$). MFR had no effect on glomerular number at birth (27.0±1.8 vs 26.5±2.1 per mm^2) or at 3 weeks (31.5±3.1 vs 32.1±2.9 per mm^2), but MFR offspring demonstrated a marked reduction (54%) by 8 weeks of age (15.9±3.6 vs 35.1±3.1 per mm2, $p<0.01$).

Conclusions: MFR has profound effects on offspring kidney structure beginning by 3 weeks of age, when the glomeruli are significantly hypertrophic (increased surface area), an effect which was sustained at 8 weeks of age. In contrast, MFR effect on glomerular number occurred between 3 and 8 weeks. These results suggest that the known effects of glomerular hypertrophy inducing glomerular sclerosis may be the putative mechanism by which MFR programs reduced glomerular number in adult offspring.

142

A Possible Role for Myostatin in Potentiating Developmental Programming. Mark P Green, Claire C Ospechook, Mark H Vickers, John J Bass, Murray D Mitchell.* *NRCGD, The Liggins Institute, University of Auckland, Auckland, New Zealand.*

Myostatin is considered to be as a negative regulator of muscle growth but it also regulates glucose metabolism and homeostasis, as well as adipogenesis. Recent studies have identified myostatin in human and murine placentae and showed myostatin to have direct effects on glucose uptake independent of insulin. Maternal undernutrition is known to have adverse effects on both fetal growth and metabolism, which can predispose the offspring to type II diabetes and obesity in adulthood. ***Objective:*** Using a model of developmental programming and postnatal high fat (HF) nutrition, we investigated placental myostatin concentration and generational effects on the phenotype of offspring. ***Methods:*** Female Wistar rats received either a standard diet *ad lib.* (AD group) or 30% of the AD group (UN group) throughout gestation. Female offspring (F1 generation) were fed the standard diet *ad lib.* until d140, mated with AD males, and fed a standard diet *ad lib.* during gestation. A cohort of pregnancies was terminated at day E20. Litter size was standardized and the F2 pups (both sexes) were nursed by their dams until weaning, producing 2 groups of offspring (AD-AD and UN-AD). Within each group and sex, F2 animals were fed either a standard or HF (65% kcals fat) diet *ad lib.* from weaning until d140. Body weights were recorded and on d130 body composition was quantified via DEXA analysis. ***Results:*** E20 pup weights were not different between treatments, however, placentae of UN-AD pups were smaller than AD-AD pups. UN-AD compared to AD-AD placentae had increased myostatin concentrations. At weaning, F2 pup body weights were not different between groups, only between sexes. On d140 UN-AD animals (both sexes) fed the HF diet were lighter and growth rates reduced compared to their AD-AD contemporaries. This was not evident in animals fed the standard diet. In HF fed animals DEXA showed a reduced percentage body fat in UN-AD compared to AD-AD animals. Feeding the HF diet to UN-AD animals (both sexes) increased plasma glucose and insulin concentrations relative to animals fed a standard diet. ***Conclusions:*** These results indicate that placental myostatin may play a role in regulating nutrient supply and thus facilitate developmental programming over generations. Higher placental myostatin may also provide resistance to diet-induced obesity, albeit at the cost of glucose homeostasis. *Supported by Maurice and Phyllis Paykel Trust.*

143

Programmed Adipogenesis Mechanisms Contributes to Offspring Obesity. Mina Desai,[1] Robert Lane,[2] Guang Han,[1] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Pediatrics, University of Utah, Salt Lake, UT, USA.*

OBJECTIVE: Maternal food restriction (MFR) during pregnancy results in growth restricted offspring which develop adult obesity. Although increased food intake contributes, in part, to enhanced weight gain, the role of peripheral adipose tissue metabolism is unknown. The development of obesity is associated with increased adipocyte differentiation and upregulation of lipogenic genes. Both glucocorticoids and insulin are potent inducers of adipocyte differentiation. The glucose transporter, GLUT4 in adipose tissue promotes glucose flux into adipocytes, which together with insulin facilitates lipogenesis. To examine peripheral mechanisms of programmed obesity, we determined the protein expression of glucocorticoid and insulin receptors, GLUT4 and leptin in 9 month old Control and MFR offspring.

STUDY DESIGN: Control dams (n=6) received ad libitum food, whereas study dams were 50% food-restricted (n=6) from pregnancy day 10 to 21. 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. At 9 months of age, adipose tissue was analyzed for protein expression of glucocorticoid and insulin receptors, GLUT4 and leptin (Western Blot).

RESULTS: MFR adipose tissue showed significantly increased glucocorticoid receptor expression (911±93 vs. 538±89 AU, $p<0.01$) whereas insulin receptor expression was decreased (553±47 vs. 1455±76 AU, $p<0.01$) as compared to Controls. GLUT4 expression was significantly increased (2570±374 vs. 1572±219 AU, $p<0.01$). Consistent with hypertrophic adipocytes, leptin expression was also significantly increased (1187±59 vs. 803±103 AU, $p<0.01$).

CONCLUSIONS: Upregulation of glucocorticoid receptor and GLUT4 may contribute to facilitate adipocyte differentiation and lipogenesis in MFR offspring, resulting in increased adipose tissue leptin expression. Together with prior studies, these results suggest that MFR-induced programmed obesity may result from both increased food intake and enhanced adipogenesis.

144

Programmed Upregulation of Adipogenic Transcription Factors in Intrauterine Growth Restricted Offspring Adipocytes: Effect of PPARγ Modulators. Mina Desai,[1] Robert Lane,[2] Guang Han,[1] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Pediatrics, University of Utah, Salt Lake, UT, USA.*

OBJECTIVE: Key adipogenic transcription factors include peroxisome proliferator-activated receptor gamma 2 (PPARγ2), CCAAT/enhancer binding protein-α (C/EBPα), and sterol regulatory element binding protein 1 (SREBP1). PPARγ2 acts synergistically with C/EBPα to activate SREBP1, which promotes lipogenesis in adipose tissue. Maternal food restriction (MFR) during pregnancy results in increased adipose tissue PPARγ2, C/EBPα and SREBP1 mRNA and protein expression, potentially contributing to the development of offspring obesity. We sought to determine if increased adipogenicity was an acute, short term effect or a programmed response in MFR offspring. We further examined the adipocyte response to PPARγ agonist (rosiglitazone) and antagonist (BADGE).

STUDY DESIGN: Control dams received ad libitum food (n=6), whereas study dams were 50% food-restricted (n=6) from pregnancy day 10 to 21. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by Control dams and at 3 weeks of age, adipose tissue was obtained and primary adipocyte cell culture was established. Following 100% confluence, MFR and Control adipocytes were treated to two doses (1 and 10μm) of either rosiglitazone or BADGE for 24h. PPARγ2, C/EBPα, and SREBP1 mRNA levels were analyzed and compared to the respective untreated cells.

RESULTS: MFR adipocytes had significantly increased mRNA expression of PPARγ, C/EBPα and SREBP1 as compared to Control adipocytes. Rosiglitazone treatment at lower dose (1μm) was sufficient to increase PPARγ2 (6-fold) and SREBP1 (2-fold) in Control adipocytes, though there was no effect on C/EBPα. Conversely, MFR adipocytes required higher dose of rosiglitazone (10μm) to upregulate PPARγ2 (4-fold) and C/EBPα (2-fold), though no effect was seen on SREBP1. BADGE at 1 and 10μm concentration reduced the expression of PPARγ2 and SREBP1 in Control adipocytes but had no effect on MFR adipocytes.

CONCLUSIONS: MFR adipocytes demonstrate programmed upregulation of adipogenic transcription factors PPARγ, C/EBPα, and SREBP1 which promote obesity. The markedly reduced response to PPARγ modulations further indicates impaired adipogenic regulation. These findings suggest that IUGR offspring may not respond to traditional pharmacologic approaches to obesity.

145

Gender Specific Programmed Hepatic Lipid Dysregulation in Intrauterine Growth Restricted Newborn. Gyu Y Choi,[1] Guang Han,[1] Darran N Tosh,[2] Roy Z Mansano,[1] Michael G Ross,*[1] Mina Desai.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Adelaide, Australia.*

Objective: Transcription factors, peroxisome proliferators-activated receptors (PPARα, PPARγ) and sterol response element-binding protein (SREBP1) regulate lipid homeostasis. PPARγ and SREBP increase expression of the lipolytic enzyme lipoprotein lipase (LPL) and the lipogenic enzyme fatty acid synthase (FAS). We have previously shown that 25% maternal food restriction during pregnancy results in IUGR offspring with smaller hepatic lobule size, and gender specific elevated content of triglyceride and cholesterol in males, and decreased cholesterol in females. We sought to determine if hepatic expression of lipid homeostatic transcription factors and key enzymes explains the gender specific cholesterol/triglyceride responses.

Method: From day 10 to term gestation, control pregnant rats received ad libitum diet (n=6), whereas study rats were 25% food-restricted (MFR; n=6). Following delivery, all dams received ad libitum diet throughout lactation and nursed their own pups. At birth, litter size was culled to 4 males and 4 females. At 21 days of age, liver from 1 male and 1 female per litter was analyzed for mRNA levels of PPARα, PPARγ, SREBP, LPL and FAS using real-time RT-PCR. Data is presented as fold difference normalized to GAPDH. Values shown are mean ± SE.

Results: MFR males had significantly increased mRNA expression of SREBP1 (2-fold, $p<0.01$) as compared to controls, though no change was seen in PPARα and PPARγ. Further, MFR male expression of LPL was increased (7-fold, $p<0.01$), though there was no change in FAS. Conversely, MFR females showed no change in SREBP1 expression whereas decreased expression was evident in PPARα (0.6-fold, $p<0.05$) and PPARγ (0.1-fold, $p<0.001$) as compared to control females. The expression of LPL and FAS in MFR females was comparable to controls.

Conclusion: MFR during pregnancy results in gender specific changes in lipid profile. MFR males show elevated hepatic triglyceride and cholesterol with upregulation of SREBP1 and LPL, indicating enhanced lipogenesis. MFR females exhibit decreased hepatic cholesterol with downregulation of PPARα and PPARγ, suggesting no adverse effect of hepatic lipid profile. Thus, developmental programming occurring during the gestational period results in sex dependent altered lipid metabolism and increased risk of cardiovascular disease in male offspring.

146

Programmed Hepatic Glucose Metabolism in Intrauterine Growth Restricted Newborn: Gender Specific Contribution to Hypoglycemia. Gyu Yeon Choi,[1] Glenda Calvario,[1] Ambica Garg,[1] Roy Mansano,[1] Mina Desai,[1] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]UC Irvine, Irvine, CA, USA.*

OBJECTIVE: IUGR offspring are at increased risk of newborn hypoglycemia, presumed secondary to reduced placental glucose transfer. Glucose homeostasis is regulated primarily by the liver, via key hepatic glycolytic (glucokinase; GK) and gluconeogenic enzymes (phosphoenopyruvate carboxykinase; PEPCK and glucose-6-phophatase; G-6-Pase). We have previously shown that maternal food restriction (MFR) during pregnancy results in IUGR offspring with smaller hepatic lobule size and relative hypoglycemia through 3 wks of age. We hypothesized that hepatic glycogen content and the expression of hepatic enzymes involved in glucose metabolism of IUGR offspring would reflect a programmed carbohydrate dysfunction.

METHOD: From day 10 to term gestation, control pregnant rats received ad libitum diet (n=6), whereas study rats were 25% food-restricted (MFR; n=6). Following delivery, all dams received ad libitum diet throughout lactation and nursed their own pups. At birth, litter size was culled to 4 males and 4 females. At 3 wks, liver from 1 male and 1 female per litter was analyzed enzymatically for hepatic glycogen and for mRNA levels of PEPCK, GK and G-6-Pase using real-time RT-PCR. Data is presented as fold difference normalized to GAPDH. Values shown are mean ± SE.

RESULTS: At 1d of age, MFR pups had lower body weight (6.3±0.1 vs 7.1±0.2 g, $p<0.01$) with increased relative liver weight (4.6±0.1 vs 4.2±0.1%, $p<0.01$). At 3 wks, MFR offspring had body and liver weights comparable to the controls. However, MFR females exhibited significantly decreased hepatic glycogen (0.8±0.1 vs 1.6±0.3 μmol/g liver, $p<0.01$) as compared to control females. Further, mRNA expression of G-6-Pase was significantly decreased in MFR females (0.2-fold, $p<0.01$) as compared to control females. No change was evident in PEPCK or GK expression. Conversely MFR males exhibited no change in any of the parameters studied.

CONCLUSION: MFR during pregnancy results in IUGR offspring with gender specific changes in glucose homeostasis. Reduced hepatic glycogen content in 3 wk old MFR females is associated with reduced gluconeogenesis enzyme expression (G-6-Pase). We propose that programmed downregulation of gluconeogenic enzymes contributes importantly to IUGR newborn hypoglycemia.

147

Placental Insufficiency Programs Regional Differences in Vascular Function and Passive Mechanical Wall Properties in One Year Old Female Rat Offspring. Marc Q Mazzuca,[1] Mary E Wlodek,*[1] Helena C Parkington,[2] Julie A Owens,[3] Marianne Tare.[2] *[1]Physiology, Univ of Melbourne, Melbourne, VIC, Australia; [2]Physiology, Monash Univ, Melbourne, VIC, Australia; [3]Obstetrics and Gynaecology, Univ of Adelaide, Adelaide, SA, Australia.*

OBJECTIVE: Low birth weight has been associated with increased risk of developing cardiovascular disease in adulthood. Previously we have found that male offspring exposed to placental insufficiency had hypertension, altered vascular reactivity and passive mechanical wall properties at 6 months of age. In this study we examined the influence of placental insufficiency on vascular smooth muscle, endothelial function and passive mechanical wall properties of female offspring at 1 year of age.

METHODS: On day 18 of gestation bilateral uterine vessel ligation (Restriction, R) or sham surgery (Control, C) was performed in WKY rats. Female offspring were killed at one year of age. Mesenteric, renal, femoral and uterine arteries were mounted on wire and pressure myographs to assess smooth muscle and endothelial function and passive mechanical wall properties, respectively.

RESULTS: R did not alter mean arterial pressure in adult female offspring (C, 121±3 *vs* R, 126±2 mmHg, *n=5* each). Uterine arteries from R rats were more sensitive to endothelial agonist acetylcholine, but maximal relaxation was similiar. However, relaxation attributed to endothelium-derived hyperpolarizing factor (EDHF) in uterine arteries was significantly reduced in R rats. Endothelium-dependent relaxation in all other arteries was unaffected. Smooth muscle contraction to phenylephrine and relaxation to sodium nitroprusside was not different (R *vs* C) for all arteries. Passive mechanical wall properties were significantly altered (R *vs* C) in uterine, femoral and renal but not mesenteric arteries. Vessel wall stiffness was increased (R *vs* C) in uterine and femoral whereas it was decreased in renal arteries.

CONCLUSIONS: Placental insufficiency impaired EDHF-mediated relaxation in uterine arteries whereas reactivity to vasoconstrictors and vasodilators was preserved in all other vascular beds. In contrast, placental insufficiency had more widespread effects on passive mechanical wall properties across the vasculature. Placental insufficiency in female offspring programs region specific alterations in vascular properties possibly influencing cardiovascular function, and may restrict uterine blood flow during pregnancy altering intrauterine development of the next generation.

148

Evaluation of *Mecp2$^{R308/Y}$* Mutant Mice as a Model To Study Fetal Programming. Zhiyin Yu,[1] Rebecca Rosetta,[1] Haleh Sangi-Haghpeykar,[1] Ignatia Van den Veyver.*[1,2] *[1]Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.*

Objective: DNA (CpG) methylation is altered at candidate loci after prenatal modification of dietary methyl donor supply. *Mecp2$^{R308/Y}$* mutant mice, with a truncating mutation of the methyl-CpG-binding protein 2 gene which models Rett syndrome, have increased weight gain on a high methyl donor diet. Rett syndrome patients have subtle changes in glucose metabolism. To investigate whether *Mecp2$^{R308/Y}$* mice are a sensitive model for epigenetic modification of weight and glucose tolerance (GT), we fed them three different methyl-donor diets and studied their weight and glucose tolerance.

Methods: Female 129Sv/Ev *Mecp2$^{R308/X}$* mice were fed diets with high (HM), regular (RM) or low (LM) methyl donor content starting 4 weeks prior to mating to wt males; offspring received the same diets. *Mecp2$^{R308/Y}$* and wt male offspring were compared. Diets were continued during lactation and in offspring and were based on the Purina 5001 rodent diet; the HM diet contained 22 mg/kg folic acid, 15g/kg betaine, and was supplemented with vitamin B12 and zinc; the LM diet contained 1.3 mg/kg folic acid, without other supplements. Male offspring were weighed weekly until 12 weeks, then monthly until 1 year of age. Intraperitoneal glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed at 6 months and 1 year (n=9-11 per group) on wt and *Mecp2$^{R308/Y}$* mice. Statistical analysis was by T-test and ANOVA (significance set at $p<0.05$).

Results: At 6 months *Mecp2$^{R308/Y}$* mice are heavier than wt mice on all diets, the effect was biggest for HM diet: HM: 28.9+/-4.7gr vs. 24.3+/-1.7gr (n=18 each), p=0.0004; LM: 25.7+/-1.7gr vs. 23.4+/-1.8gr (n=19 and 18), p=0.0002 and RM: 25.9+/-3.5gr vs. 23.5+/-2.5gr, p=0.017 (n=19 and 21). There was no weight difference at 1 year. *Mecp2$^{R308/Y}$*, but not wt mice have impairment of glucose tolerance at 6 months compared to 1 year on HM (p=0.0009) and LM (p=0.004), but not on RM diet. At 6 months, *Mecp2$^{R308/Y}$* mice on HM (p=0.03) and LM (p=0.007) diet had impaired GT compared to wt mice. There were no significant GTT or ITT differences at 1 year.

Conclusions: We confirmed that 129/SvEv *Mecp2$^{R308/Y}$* mice are heavier than wt mice and have diet-dependent impairment in glucose tolerance at 6 months. This suggests that these mice are a good model to study fetal programming.

149

Maternal Diet Induced Obesity and Programming of the Metabolic Syndrome. Celine Morens,[1,2] Brigitte Reusens,[1] Aldert Piersma,[3] Claude Remacle,[1] Frans A Van Assche.*[2] *[1]Catholic University of Louvain, Louvain-la-Neuve, Belgium; [2]KULeuven, Leuven, Belgium; [3]RIVM, Bilthoven, Netherlands.*

Objetives: With the current worldwide obesity epidemic, pre-gravid obesity is more and more common, so that more and more pregnant women are also obese. Epidemiological studies have suggested a correlation between pre-pregnancy maternal body mass index and increased risk of obesity in children and young adults.

Our current studies aim at determining, in a rodent model, whether maternal diet-induced obesity (DIO) could « program » the development of the metabolic syndrome (MetS) (and especially obesity and insulin resistance) in the offspring.

Methods: We developed a model in which female Wistar rats are rendered obese by consuming, from weaning on, a diet enriched in fat and/or sucrose (OB1: 45% kcal fat, 15.5% kcal sucrose, and OB2: 45%kcal fat + sweetened condensed milk). A group of females fed lab chow (C) was also included as control. At 3 months of age, females were mated and allowed to deliver spontaneously. At 21 days of age, their offspring were weaned onto usual lab chow diet. Body weight, food intake, plasma parameters, insulin resistance (hyperinsulinemic-euglycemic clamps) and body composition were assessed in both male and female progeny.
Results: The offspring of the DIO dams had significantly lower birth weights (C: 6.9±0.2; OB1: 6.5±0.2 and OB2: 6.1±0.2 g; Anova P=0.0002). However, during lactation, their weight caught up so that, at weaning, they were significantly heavier than the C offspring (C: 56.8±1.7; OB1: 69.9±2.4 and 68.1±5.0g, Anova P=0.0008). After weaning, the weight gain of the OB1 and OB2 offspring remained higher and, at 3 and 6 months of age, both males and females presented an increased body weight, with an increased accumulation of body fat (measured at 6 months). This was, in part, due to an increased food intake, especially in males. At 3 months (males) and 4 months (females) of age, insulin resistance was assessed in the offspring. Animals were submitted to a hyperinsulinemic-euglycemic clamp (insulin infusion rate = 5 $mU.kg^{-1}.min^{-1}$). PRELIMINARY data show that insulin sensitivity, as measured by the Glucose Infusion Rate (GIR, mg $glucose.kg^{-1}.min^{-1}$), tended to be reduced in the OB1 and OB2 offspring (especially in the males). Experiments are now running to confirm those results.
Conclusion: Our results are in line with the idea of a programming of the MetS by obese dams.
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150

Outcomes of Sensitization Are Expressed Differently in Prenatally and Actively Sensitized Male, but Not Female Guinea Pigs. Egle Bytautiene, Phyllis K Gamble, Monica Longo, Gary D Hankins,* Garland D Anderson, George R Saade. *Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Objective: Previously we have shown that maternal exposure to allergens during pregnancy alters immune responses in the offspring later in life. The objective of this study was to determine if responses differ in prenatally sensitized and actively sensitized guinea pigs.
Study design: Three months old non-pregnant female and male guinea pigs were actively sensitized with ovalbumin (100 µg) and aluminum hydroxide (100 mg) in saline (active group). Pregnant female guinea pigs were sensitized in the same fashion on days 30-35 of gestation and their offspring followed until 3 months of age (prenatal group). Tracheal rings were then obtained from male and female animals in both groups and mounted for isometric tension recording in organ chambers filled with Kreb's buffer. During a 1- to 2-hour equilibration period, tension was gradually adjusted to 2 g. Rings were repeatedly pre-contracted with 60 mM potassium chloride until stable contraction was obtained. Rings were challenged with ovalbumin (150 µg/mL). The other set of tracheal rings was used to obtain concentration-response curves to histamine (10^{-9} – 10^{-4} M) .Contractile responses were calculated as a percentage of the reference contraction to potassium chloride, and Student's t test was used for statistical comparisons ($p<0.05$)
Results: Responses to ovalbumin were significantly higher in males born to sensitized mothers when compared with actively sensitized males. Increase in tension induced by histamine at the highest concentration was significantly higher in prenatal versus active sensitization in males. There were no differences observed between prenatally and actively sensitized female guinea pigs.
Conclusions: Hypersensitivity responses are more pronounced with prenatal sensitization only in male animals. We propose that manifestations of type I hypersensitivity reaction, including, but not limited to asthma, depend on the timing, route of initial exposure to antigen, and the gender, and further investigations into these mechanisms could direct research efforts into preventative and therapeutic strategies.

151

Small for Gestational Age and Fetal Growth Restriction Very Low Birth Weight Neonates Have Different Postnatal Growth Curves. Tullia Todros,*[1] Vanessa Ciriminna,[1] Enrico Bertino,[2] Gessica Rossetti,[2] Manuela Mensa,[1] Alessandra Coscia,[2] Claudio Fabris,[2] Elena Spada,[3] Silvano Milani.[3] *[1]Obstetrics and Gynecology, University of Turin, Italy; [2]Neonatal Unit of Pediatric Sciences, University of Turin, Italy; [3]Institute of Medical Statistics and Biometry, University of Milan, Italy.*
Objective: to investigate the impact of intrauterine fetal growth pattern on postnatal growth velocity of very low birth weight (VLBW) infants.
Methods: Intrauterine and postnatal growth to over 2 years of age was assessed in 157 VLBW infants (birth weight between 500-1500g) born at Maternal Fetal Medicine Unit and admitted to Neonatal Unit between January '94 and December '99. Inaccurate estimate of gestational age, multiple pregnancy, congenital anomalies, death and loss to follow-up were excluded. We identified six distinct subgroups of VLBW neonates. According to *birth weight* (BW) we defined SGA neonates as BW < 10°centile and AGA as BW between 10°-90° centiles. According to *fetal ultrasound biometry* we defined SGA and AGA as abdominal circumference (AC) < and > 10° centile respectively assessed on distance charts. According to *fetal biometry and Doppler velocimetry of umbilical artery* we defined FGR as AC <10° centile with abnormal Doppler velocimetry and AGA as AC>10° with normal Doppler velocimetry.
Body weight was recorded daily up to 28 days, weekly up to discarge, then at 1, 3, 6, 9, 12, 18 and 24 months of age. Individual growth profiles were fitted with a seven constant, exponential-logistic function suitable for modelling weight loss and weight recovery, two peaks, and the subquent slow decrease in growth velocity.
Results: *According to birth weight*: SGA had a growth velocity rate lower than AGA until 12 months and significantly lower between 12 and 24 months of corrected age.
According to fetal biometry: SGA had a growth velocity rate lower than AGA until 1 year of age and the same growth velocity rate afterwards.
According to *fetal biometry and Doppler velocimetry:* FGR had growth velocity slightly lower until 15 weeks of postnatal age and higher afterwards.
Conclusions: Fetal biometry associated with Doppler velocimetry identifies a subgroup of SGA neonates who suffered growth restriction in utero and have different (catch-up) growth during the first 24 months of age. Therefore it is useful tool not only in fetal surveillance and in timing of delivery, but also to identify subjects who need different neonatological, nutritional and medical care.

152

Impact of Maternally Administered Endothelin Receptor Antagonism on Rat Pup Survival and Growth. Larry G Thaete,* Sylvia Synowiec, Mark G Neerhof.* *Obstetrics & Gynecology, Evanston Northwestern Healthcare and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.*
Background: Endothelin receptor A (ET_A) antagonism has been shown to normalize fetal growth in several models of fetal growth restriction in the rat. However, direct administration of ET_A antagonists to newborn rats within 3 hours of delivery has been consistently associated with neonatal demise, raising concerns about the safety of their use late in pregnancy. Perinatal exposure to ET_A antagonists (maternal administration in late gestation) and its impact on rat pup survival and growth has not been investigated.
Objective: To determine the impact of an ET_A antagonist on pregnancy outcome, survival and growth of rat pups.
Methods: Timed pregnant Sprague-Dawley rats were treated with FR139317 (12 mg/kg/day; ET_A antagonist) or vehicle, by subcutaneous osmotic pump connected to an intravenous catheter, from gestational day 14 (term=22 days) through parturition. Five pregnant rats in each group were allowed to deliver spontaneously and to nurse their pups through post-partum day 7. Viability of newborns, litter sizes, and pup weights were recorded at birth and at day 7. Results are presented as means ± SE.

Results:

Pregnancy Outcome, Survival and Growth of Rat Pups with Maternal ET_A Antagonism

Group	Gest Age at Birth (days)	Litter Size (# pups)	Live Pups at Delivery	Live Pups at 7 Days	Birth Weight (g)	Weight at 7 Days (g)
Vehicle	22.2 ± 0.40	11.8 ± 0.17	11.0 ± 0.45	10.5 ± 0.87	5.5 ± 0.19	15.4 ± 1.94
FR139317	22.6 ± 0.24	13.0 ± 0.89	12.2 ± 1.16	12.2 ± 1.16	5.6 ± 0.15	14.6 ± 0.47

There were no statistically significant differences in any of these parameters between groups.

Fetal and Neonatal Loss with Third Trimester Maternal ET_A Antagonism

Group	% Stillbirth	% Neonatal Loss
Vehicle	6.9 ± 4.0[a]	8.3 ± 8.3[b]
FR139317	6.4 ± 4.5[a]	0.0 ± 0.0[b]

[a]Vehicle group: One stillbirth in each of two rats and three stillbirths in one rat. FR139317 group: One stillbirth in one rat and three stillbirths in one rat. [b]One vehicle-treated rat lost four neonates; no neonatal losses with FR139317.

Conclusions: Maternal administration of an ET_A antagonist from gestational day 14 through parturition has no adverse impact on survival or growth of rat pups. The next experimental step toward considering this intervention in human pregnancy is to demonstrate equivalent safety of ET_A antagonism while treating fetal growth restriction.

153

Being Born Small or Accelerated Postnatal Growth Programs Decreased Adult Bone Growth and Strength. Tania Romano,[1] John D Wark,[2] Julie A Owens,[3] Howard A Morris,[4] Mary E Wlodek.*[1] *[1]Physiology, Univ of Melbourne, Parkville, VIC, Australia; [2]Medicine, Royal Melbourne Hospital, Univ of Melbourne, Parkville, VIC, Australia; [3]Obstetrics & Gynaecology, Univ of Adelaide, Adelaide, SA, Australia; [4]Hanson Institute, Adelaide, SA, Australia.*

Objectives: Intrauterine growth restriction and its associated accelerated postnatal growth increase the risk of developing adult diseases, including osteoporosis. We have shown that placental restriction impairs pre- and postnatal nutrition in the rat. This study used cross-fostering to assess the influence of the prenatal and postnatal environment on adult bone size and strength.

Methods: Bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) was performed on day 18 of gestation in WKY rats. Control, Reduced (RED; reducing litter size of C on day 1 to match R) and Restricted pups were cross-fostered onto C or R mothers 1 day after birth. Male offspring weight (birth to 6 months) and femur length and biochemical markers of bone turnover (6 months) were measured. Global femur mineral content was quantified (DXA) and cortical and trabecular bone area, circumference, density, content, cortical thickness and estimated bone bending strength determined by pQCT at 6 months.

Results: Restricted male pups were born lighter than Controls and remained smaller than C-on-C pups at 6 months. Reduced litter pups of normal body weight grew slowly during lactation then accelerated their growth after weaning when suckled on a R mother (RED-on-R). Restricted (R-on-R, R-on-C) and RED-on-R pups had shorter femurs with reduced bone area, cortical thickness, periosteal and endosteal circumferences, as well as bone bending strength (~20%) compared to C-on-C. Cross-fostering a R male pup onto a mother with normal lactation (R-on-C) did not improve postnatal growth nor restore bone parameters. Bone mineral density and biochemical bone markers were similar across all groups.

Conclusions: Being born small (R-on-R, R-on-C) or being born of normal weight but having slowed growth followed by accelerated growth after weaning (RED-on-R) reduced bone dimensions and bending strength. Correcting the poor lactational environment for small pups did not improve postnatal growth, bone growth or strength, suggesting the intrauterine environment is critical for bone programming in males. Being born small, or accelerated growth after weaning programs reduced bone bending strength and may increase osteoporotic bone fracture risk in adult life.

154

Does Umbilical Artery Extracellular Matrix Gene Expression Differ between Small for Gestational Age and Appropriately Grown Fetuses? Wendy Kinzler,*[1] Siew Choon Tee,[1] Morgan Peltier,[1] John Smulian,*[1] Marion Gordon.[2] *[1]Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA; [2]Pharmacology and Toxicology, Rutgers, The State University of New Jersey, Piscataway, NJ, USA.*

Background: Previous work has demonstrated differences in umbilical artery extracellular matrix components among preterm growth restricted and appropriately grown fetuses -identifying a possible link to altered vascular function.

Objective: To determine if the expression of various extracellular matrix components within the umbilical arterial wall of term pregnancies differs amongst small for gestational age (SGA) and appropriate for gestational age (AGA) fetuses.

Methods: The umbilical cords from term pregnancies complicated by estimated fetal weights < 10th percentile with (n=5) or without (n=13) abnormal umbilical artery velocimetry were collected at the time of delivery for comparison with appropriately grown controls (n=12). The umbilical artery was dissected free from the Wharton's jelly and immediately placed into RNALater and stored at -70 ^{o}C. Total RNA was purified using the Trizol reagent. Relative levels of mRNA for extracellular matrix components were evaluated using real-time RT-PCR. Levels of expression for collagen types I, III and XIV and the proteoglycan decorin were normalized to GAPDH. Data were analyzed using SAS and $P<0.05$ was considered statistically significant. Results are presented as least-squares means ± SEM for number of cycles to amplification (which is inversely proportional to the amount of RNA template).

Results: mRNA levels for collagen III and decorin did not differ between groups. SGA pregnancies demonstrated decreased gene expression for collagens I ($P=0.002$) and XIV ($P=0.032$) compared with AGA pregnancies. Among SGA infants, expression of these genes did not differ between those with normal vs. abnormal umbilical artery Doppler velocimetry.

Conclusions: Umbilical arteries from small for gestational age fetuses have decreased expression of collagen types I and XIV that is unaffected by umbilical artery Doppler status.

155

Effects of Sustained Release Growth Hormone on Lambs at Birth. Jill M Koch, Matthew E Wilson. (SPON: Ronald R Magness). *Animal and Nutritional Sciences, West Virginia University, Morgantown, WV, USA.*

The onset of adult diseases such as type II diabetes, obesity, heart disease and renal failure are associated with detrimental uterine environment during fetal development. While several groups are investigating the effects of a detrimental environment we have recently been investigating methods to provide a positive environment with hopes of ameliorating the negative consequences later in life. Altering the early embryonic environment may lead to late gestation alterations in organ and body development. The objective of this study was to determine effects of a single treatment of sustained release growth hormone on the lambs at birth. Ewes were synchronized using two injections of prostaglandin 8 days apart and the day of the second injection those ewes assigned to treatment received 500 mg of sustained release growth hormone. All ewes were penned with a fertile ram. Upon lambing, all lamb birth weights, crown rump lengths and abdominal girths were determined and a serum sample collected to determine IGF-1 concentrations. Ram lambs were then euthanized to determine liver, heart and brain weights. A portion of the liver was snap frozen and used to determine IGF-1 and GH receptor mRNA utilizing real-time PCR. A portion of the base of the ventricles was removed to determine left and right ventricular wall thickness. Treated ewes gave birth to lambs with a heavier birth weight compared to control lambs (4.67 ± 0.07 vs. 4.08 ± 0.21 kg; $P< 0.05$). Lambs from treated ewes also had greater abdominal girth (31.6 ± 1.1 vs. 29.9 ± 0.7 cm; $P< 0.05$). However, there was no difference in crown rump length (50.2 ± 1.2cm). Liver IGF-1 and growth hormone receptor message were reduced ($P < 0.05$) in lambs gestated by ewes treated with growth hormone compared to controls. The left ventricular wall was thinner in hearts from lambs gestated in growth hormone treated ewes compared to those from controls. There was a positive correlation between lamb body weight and serum IGF-1 ($r=0.48$; $P< 0.05$). In conclusion, treating ewes with sustained release growth hormone not only increased lamb body weight but also resulted in alteration in the growth and development of the lambs.

156

Endometrial Cancer Cells Treated with LPA Increase Activation of MMP-2. Leslie R Boyd, Edgardo V Ariztia, David Fishman.* *Dept. of Gynecologic Oncology, New York University School of Medicine, New York, NY, USA.*

Objectives: Matrix metalloproteinases (MMPs) are responsible for the breakdown of extracellular matrix components, and are important in the metastatic phenotype. HEC-1A is an endometrial cancer cell line, derived from a moderately-differentiated tumor. These cells do not produce MMP-2, an important mediator of invasive behavior. We sought to: (1) evaluate if HEC-1A cells have the capacity to activate exogenous proMMP-2; (2) determine if lysophosphatidic acid (LPA), an activator of MMPs in other cancer cell lines, would enhance this conversion; and (3) determine if MMP-2 activation is mediated through the recruitment of MT1-MMP.

Methods: HEC-1A cells were maintained as per known protocols. Conditioned media from chondrosarcoma cells, a source of proMMP-2, was added to HEC-1A cells. Cells were treated with 0.1, 1.0 and 10μM LPA. Conditioned media from the cells was analyzed for the presence of MMP-2 and its proenzyme utilizing gelatin zymography. Cellular invasion through a collagen matrix was quantified using a modified Boyden chamber assay. Migration was evaluated using a wound healing assay, with quantification of wound closure at 48 hours. Immunoblotting to detect MT1-MMP was performed utilizing a polyclonal rabbit antibody against the hinge-region of MT1-MMP. mRNA was isolated from treated cells, and after cDNA production, primers to MT1-MMP were used for RT-PCR evaluation.

Results: HEC-1A cells activated exogenous proMMP-2 to MMP-2, as confirmed by gelatin zymography. Invasion of HEC-1A cells treated with proMMP-2 was significantly increased as compared to controls ($p < .001$). LPA increased invasion in cells with and without exogenous proMMP-2, with the most invasive cells being those treated with both LPA and exogenous proMMP-2. The addition of both exogenous MMP-2 and LPA significantly increased cellular migration ($p < .001$). On Western blot, MT1-MMP was visualized in all cell lysates with optimal activity noted at 1.0 μM LPA. RT-PCR to evaluate the number of MT1-MMP transcripts did not show an increase in those cells treated with LPA.

Conclusions: HEC-1A cells convert exogenous proMMP-2 to its active form, and in doing so exhibit a more invasive phenotype. LPA further increases these behaviors, and may do so by increasing translocation of MT1-MMP to the cell membrane. This may reflect the tumor microenvironment, with endometrial cancer cells responding to bioactive lipids and proenzymes secreted by the surrounding stroma.

157

Implantation Is Associated with Cell-Specific Expression of Natriuretic Peptides. Tianbing Ding, Jeff Reese,* Bibhash C Paria. *Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA.*

Context: While blastocyst implantation can occur in the absence of ovarian estrogen (E) in the progesterone (P_4)-primed uterus of hamsters, pigs, rabbits, monkeys and possibly in humans, it does not occur in mice and rat without ovarian E. Thus, the hamster as an animal model for P_4-dependent implantation could likely be exploited to identify uterine receptivity and implantation markers that can be used in humans. As a step towards achieving this goal, we performed a heterologous microarray using mouse genechips to assess changes in mRNA expression patterns between the day 5 uterine implantation and interimplantation sites in hamsters. Up- and down-regulations of a number of known and unknown genes were detected at implantation sites. One of these upregulated genes was the gene encoding C-type natriuretic peptide (CNP).

Objective: CNP is the third member of the natriuretic peptide (NP) family. Two other important members of this family are atrial NP (ANP) and brain NP (BNP). The aim of this study was to characterize the expression pattern of *CNP*, *BNP* and *ANP* genes at the implantation site of hamsters and mice.

Methods: The cell-type specific expression of *CNP*, *BNP* and *ANP* were evaluated by in situ hybridization at day 5 implantation sites of hamsters and mice.

Results: We first verified CNP expression at day 5 implantation sites of hamsters and noted that *CNP* mRNAs were expressed in stromal cells immediate to, but not away from, the implanting blastocyst. We next wanted to verify interspecies differences using the mouse, and noted a similar pattern of increase in *CNP* expression at the day 5 implantation site of both the mouse and hamster. We observed no expression of *ANP* and *BNP* at day 5 implantation sites of hamsters. However, in mice while *ANP* was strictly expressed in the inner circular muscle layer of the day 5 implantation site, *BNP* was expressed in the blastocyst.

Conclusion: This is the first demonstration that natriuretic peptides are expressed at the implantation site. Since natriuretic peptides share each others functions, expression of *CNP*, *BNP* and *ANP* in three different locations in day 5 implantation sites of mice suggests their cooperative function during implantation. However, sole stromal expression of *CNP* at the day 5 implantation site of hamsters is not only an example of species-specific variation in gene expression but also suggests an exclusive role of CNP in the P_4-dependent implantation process of hamsters.

158

Uterine Phenotype in Double Versus Single LH Receptor and FSHβ Subunit Knockouts. Jing Lin, Zhenmin Lei, Xian Li, CV Rao.* *Ob, Gyn & Women Health, University of Louisville Health Sciences Center, Louisville, KY, USA.*

Introduction: Numerous studies support that uterus is a direct functional target of LH and hCG regulation. Comparatively, there is less data which suggest that FSH may also directly regulate uterus. We investigated uterine phenotype in double (DKO) versus single LH receptor (LHRKO) and FSHβ (FSHβKO) knockouts to determine the importance of LH and FSH signaling in uterine morphology.

Methods: Double knockouts were generated from previously made corresponding single knockouts by mating heterozygous LHRKO females with heterozygous FSHβKO males. The zygosities were determined by PCR of genomic DNA. Radioimmunoassays for serum hormone levels, RT-PCR, computerized video based quantitative morphometry and immunocytochemistry were used.

Results: RT-PCR and immunocytochemistry demonstrated the presence of FSH receptors in the uterus of all genotypes. Serum LH, FSH, estradiol and progesterone levels in DKO mice were similar to those of parental animals. The decrease in uterine weight, endo and myometrial thickness and luminal epithelial cell heights in DKO mice were more in line with LHRKO than with FSHβKO animals. The number of mature endometrial glands dramatically decreased in DKO mice, which resembled LHRKO mice. The number of immature endometrial glands and PCNA immunostaining, indicating the proliferative state, on the other hand, were higher in DKO than in LHRKO mice.

Conclusion: DKO mice have an additional uterine phenotype compared with LHRKO mice, which suggests that FSH may also play a role in maintaining normal uterine morphology.

159

mTOR and Glut1: Metabolically Targeted Biomarkers in Endometrial Carcinoma. Jeness M Connell,[1] Jennifer Rhode,[2] Kent Griffith,[3] Michael Rhode,[4] Malti Kshirsagar,[4] Paul Loar,[2] JR Liu,[2] Timothy R Johnson.*[1] *[1]Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA; [2]Gynecologic Oncology, University of Michigan, Ann Arbor, MI, USA; [3]Biostatistics, University of Michigan, Ann Arbor, MI, USA; [4]Pathology, University of Michigan, Ann Arbor, MI, USA.*

Objectives:

The prognosis for metastatic or recurrent type I (endometroid) or type II (non-endometroid) endometrial cancer is poor. The identification of molecular alterations in these tumor types may help redefine diagnosis, establish accurate prognosis, and may serve as novel therapeutic targets. We hypothesized that the differential metabolic phenotype observed between malignant and non-transformed cells may constitute a biochemical basis for therapeutic intervention. Increased glucose uptake is one of the major metabolic changes found in malignant tumors, a process that is mediated by glucose transporters such as Glut1. In addition, cellular growth can be regulated by mTOR (mammalian target of rapamycin) in response to the nutrient milieu. Glucose analogs and inhibitors of mTOR are currently in clinical trials in other tumor types. In this study, we sought to determine if type I and/or type II endometrial carcinomas overexpress Glut1 and/or phosphorylated mTOR (pmTOR).

Methods: We constructed three tissue microarrays (TMAs) using 75 cores from 42 patients with type I endometrial cancer, 91 cores from 34 patients with type II endometrial cancer, and 20 cores from benign endometrium. The TMAs were immunostained using polyclonal antibodies to Glut1 and pmTOR. Cytoplasmic pmTOR expression and membranous Glut1 expression was scored as negative, weak, moderate, or strong by a previously validated scoring schema.

Results: Cytoplasmic pmTOR was expressed strongly in both types I and II endometrial carcinoma (96 and 98% respectively). Membranous Glut1 was strongly overexpressed in 82% of type I and 55% of type II endometrial carcinomas and as compared to 0% of benign endometrial samples ($p<.001$).
Conclusions: Glut1 and pmTOR are highly overexpressed in endometrial carcinomas. Expression of these metabolically targeted biomarkers may identify patients who may benefit from adjuvant treatment with mTOR inhibitors and/or glucose analogs.

160

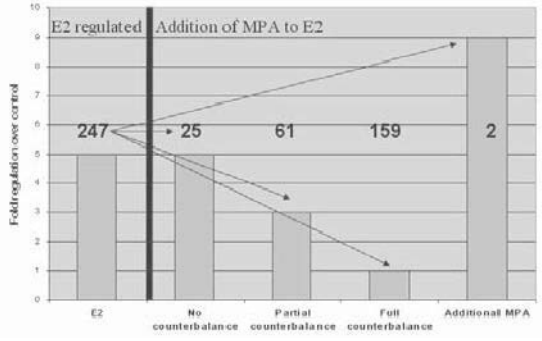
Role of Protein Kinase C in the Regulation of Estrogen Receptor Expression and Cell Morphology in HEC-1B Human Endometrial Cancer Cells. James deVente,* Patti Shaver. *Obstetrics and Gynecology, Brody School of Medicine at East Carolina University, Greeville, NC, USA.*
Background: Expression of estrogen receptor (ER) is a positive prognostic factor in breast and endometrial carcinoma and is associated with a more differentiated and less biologically aggressive phenotype. Studies have implicated the protein kinase C (PKC) family in mediating ER expression in breast cancer. In the following study we evaluate the role of PKC in mediating ER expression in the moderately differentiated human endometrial cancer cell line.
Methods: HEC-1B cells were treated with 12-0-tetradecanoyl-phorbol acetate (TPA) in varying concentrations and for varying periods of time. The PKC inhibitor, GF109203X (GF) was added in the inhibitor studies. Cellular proliferation was evaluated via [^{3}H]-thymidine uptake assay. Cellular viability was evaluated using a flow-cytometric propidium iodide exclusion assay. Expression of PKC α, βI, βII, ε, δ, η, θ, ζ, ERα, and ERβ were evaluated via western blot analysis on whole cell extracts.
Results: Treatment of HEC-1B cells with 10 nm of TPA was associated with marked morphologic changes which could be appreciated in less than 24 hours. Control cells were cuboidal and grew in aggregates whereas TPA treated cell were stellate in shape. TPA -induce morphological changes could be blocked by the PKC inhibitor GF. TPA treatment was found to increase the percentage of viable cell in a dose dependent fashion. TPA-treatment was not associated with any marked change in proliferation. TPA treatment induced an activation dependent down-regulation of PKC α, βI, and δ in a dose and time-dependent fashion. PKC βII and ε expression actually increased with increasing TPA dose and time. PKC ζ did not demonstrate any significant changes in response to TPA. ERα expression was almost completely abrogated by TPA treatment at doses as low as 10 nM in 24 hours. The PKC inhibitor GF was able to block the TPA-induced decrease in ERα expression. ERβ demonstrated little if any changes in expression in response to TPA.
Conclusions: Our study suggests that the PKC pathway plays an important role in regulating both ERα expression and cellular morphology in endometrial cancer. These events appear to be dependent on PKC activation given our ability to block these responses with a selective PKC inhibitor. Lastly, our study implicates PKC α and/or βI as attractive candidates in mediating ERα expression in endometrial cancer.

161

Progesterone Alters the Expression of Estrogen-Metabolizing Genes in Endometrial Cancer Cells: Potential Mechanism for the Protective Effect of Progesterone Against Endometrial Cancer. Salama A Salama, Sana M Salih, Ayman Al-Hendy.* *Obstetrics and Gynecology, UTMB, Galveston, TX, USA.*
Background: Endometrial cancer ranks first in incidence and second in mortality among gynecological malignancies. While excessive exposure to unopposed estrogen is a major risk factor for endometrial cancer, progesterone counteracts the carcinogenic effect of estrogen in the endometrium. Substantial evidence suggests that cytochrome P450-mediated metabolic activation of estrogen is crucial for estrogen carcinogenicity. Estrogen metabolism involves oxidation by CYP450 to carcinogenic metabolites catechol estrogens. In extrahepatic tissues, catechol-O-methyltransferase (COMT) is a key enzyme that inactivates carcinogenic estrogen metabolites.
Objective: To investigate the effects of progesterone/progesterone receptors on the expression of CYP1B1, CYP1A1, and COMT genes in an endometrial cancer cell line.
Methods: Reporter gene assays, real time RT-PCR, and western blot analysis were preformed to assess gene expression. Adenovirus vectors over-expressing either progesterone receptor A or Progesterone receptor B were used to dissect the distinct role of specific progesterone receptor isoforms on the expression estrogen metabolizing genes.
Results: Our data demonstrated that Progesterone (10^{-7} - 10^{-6} M) increased the activity of CYP1A1-luc reporter construct by 1.6- and 2.9-fold, respectively compared with the control. Under the same treatment condition, CYP1B1-Luc reporter transactivation decreased by 2- and 3.5-fold, respectively, compared with the vehicle-treated control. Using western blot analysis and real time RT-PCR, progesterone down regulated the expression of CYP1B1 gene in a dose-dependent fashion. Our data also indicated that treatment of Ishikawa cells with progesterone up-regulates COMT gene expression. Interestingly, the two progesterone receptor isoforms have distinctive effects on COMT gene expression. While PR-A is associated with up-regulation of COMT gene, PR-B mediated down regulation of the COMT gene.
Conclusion: Our results suggest that the protective effect of progesterone against endometrial cancer is attributed, at least in part, to modulation of the expression of critical enzymes involved in estrogen metabolism.

162

How Does Progesterone Counterbalances Estradiol-Signaling in the Human Endometrium. Leen J Blok, Payman Hanifi-Moghaddam, Curt W Burger. (SPON: Eric AP Steegers). *Obstetrics and Gynaecology, Erasmus University Medical Center, Rotterdam, Netherlands.*
Objective:
During the first half of the menstrual cycle, estrogen signaling is responsible for build-up of the endometrial layer. Subsequently, progesterone levels increase during the second half of the menstrual cycle and induce differentiation of the endometrium and thus inhibit estrogen-induced proliferation. The aim of the current investigations was to reveal the molecular mechanism of progesterone's inhibition of estrogen signaling
Methods:
21 healthy, postmenopausal women were included into this observational, open, non-randomized, and controlled study. The following 21 day treatments were conducted: Control-group (n=8); Estrogen-group (n=7), 2 mg/day of estradiol; Estrogen+progestagen-group (n=6), 2 mg/day of estradiol and 5 mg/day of MPA.
Results:
It was observed that upon treatment for 21 days with estrogen alone, proliferation (measured by Ki67 staining) of the endometrium is increased by at least 9-fold, while the addition of progestagens diminished this increased proliferation almost back to control levels. Furthermore, we measured the levels of ER and PR and were unable to show profound changes in receptor expression levels indicating no progestagenic effects on that level. Next endometrial gene expression profiles were compared. A group of 247 genes was found to be significantly (measured by SAM) estrogen-upregulated (setting > 3-fold). If we take this group as representative for all estrogen regulated genes, and compare the gene expression data with the data obtained after estrogen+progestagen treatment, 10% (25) of genes are unaffected, 25% (61) are partly compensated and 65% (159) of genes are completely compensated for (are back at the control level after estrogen+progestagen treatment).



Conclusions:
Progesterone very effectively compensates for estrogen regulation of a great number of genes. Since neither the estrogen receptor nor the progesterone receptor levels are markedly affected, it is likely that the compensatory effect takes place at the promoter region of effected genes.

163

Heparanase Is Up-Regulated by Estradiol during Endometrial Hyperplasia-Carcinoma Transition. Ronit Haimov-Kochman,[*1] Ahinoam Lev-Sagie,[1] Diana Prus,[1] Michal Lichtenstein,[2] Haya Lorberboum-Galski,[2] Simcha Yagel,[*1] Israel Vlodavsky,[3] Inbar Ben-Shachar.[1] *[1]Obstetrics and Gynecology, Hadassah Hebrew University Medical Center, Jerusalem, Israel; [2]Cellular Biochemistry and Human Genetics, Medical School Hebrew University, Jerusalem, Israel; [3]Vascular and Tumor Biology Research Center, The Rappaport Institute, Technion, Haifa, Israel.*

Hypothesis: Heparanase is a mammalian endo-β-glucuronidase that cleaves heparan sulphate of the extracellular matrix. The full-length 65kDa proheparanase is activated by cleavage into a 50 kDa isoenzyme. Heparanase expression has been correlated with the metastatic potential of various tumors. The endometrial hyperplasia-carcinoma sequence is dependent on unopposed estrogen exposure. We hypothesized that heparanase may be up-regulated by estrogen and over-expressed during the endometrial hyperplasia-carcinoma transition.

Methods: Heparanase isoforms were immunodetected in formalin fixed, paraffin embedded sections from patients with the following histopathologic criteria: simple hyperplasia, complex hyperplasia with and without atypia and invasive carcinoma. Normal endometrium served as control. The heparanase proenzyme and the active isoform were detected using polyclonal rabbit anti-human heparanase antibodies LinkAb and 733pAb, respectively. Quantitative PCR was employed to detect the level of heparanase mRNA level in estrogen receptor (ER)-positive Ishikawa, and ER-negative HEC1a endometrial carcinoma cell lines exposed to increasing doses of estradiol in culture.

Results: The active isoform of heparanase was consistently detected in endometrial glands throughout the hyperplasia-carcinoma continuum, whereas the heparanase proenzyme localized to endometrial stroma. Along with the malignant transformation the presence of the heparanase proenzyme increased dramatically from none in the stroma of normal and hyperplastic endometrium to abundance in malignant tumors. Cultured Ishikawa cells dramatically increased heparanase transcript levels after exposure to estradiol whereas in HEC1a cells the heparanase transcript remained unchanged.

Conclusions: Enhanced expression of the proenzyme of heparanase in the malignant endometrium and up-regulation of the heparanase mRNA transcript by estrogen in an ER positive endometrial adenocarcinoma cell line, support the hypothesis that the expression of heparanase may be correlated with the malignant potential of endometrial hyperplasia and with the clinical course of the disease.

164

Endometrial Cancer Cells Produce Pro-Invasive Vesicles Following Treatment with LPA. Leslie R Boyd, Edgardo V Ariztia, David Fishman.[*] *Gynecologic Oncology, New York University, New York, NY, USA.*

Objectives: Membrane-derived vesicles are an important feature of malignant transformation, and their presence has been correlated in vivo with poor prognosis in patients with gynecologic cancers. Proinvasive molecules have been documented within these vesicles, including matrix metalloproteinases (MMPs), which remodel the basement membrane and promote cellular motility. However, little is known regarding the ability of endometrial cancer cells to produce vesicles. Lysophosphatidic acid (LPA) is a bioactive lipid which is elevated in the serum of patients with multiple tumors, including endometrial cancer. We investigated if LPA, a known inducer of vesicles in other cell types, could induce vesicular production in endometrial cancer cells.

Methods: HEC-1A cells, originally derived from a moderately-differentiated endometrial tumor, were maintained in a humidified environment as per known protocols. Vesicles were induced by adding fetal bovine serum or LPA at .001, .01, 0.1, 1.0 and 10 mM to starved cells for four hours. Differential centrifugation was performed on the conditioned media, with ultimate recovery of the pellet. Protein assays were performed to determine the optimal concentration of LPA. Vesicles were run in 9% and 15% gelatin zymography assays to determine the presence of several MMPs including MMP-2, MMP-7 and MMP-9. Using a modified Boyden chamber, vesicles induced by fetal bovine serum and/or 1.0 mM LPA were added to HEC-1A cells to assess in-vitro invasion through a collagen matrix.

Results: Vesicles were formed using the known protocol, with optimal yields occurring with 1.0 mM LPA. Using gelatin zymography, we documented the presence of active MMP-2 and MMP-9 within vesicles at all concentrations of LPA. MMP-7 was not visualized in any vesicular samples. The addition of vesicles significantly increased invasion of HEC-1A cells through a collagen matrix as compared to controls ($p < .005$).

Conclusions: This is the first report regarding the production of vesicles in endometrial cancer cells. Physiologic levels of LPA induce the production of membrane-derived vesicles in endometrial cancer cells in vitro. These vesicles contain proinvasive MMPs, including MMP-2 and MMP-9, and increase metastatic behavior, as based on the results of an invasion assay. Future areas of investigation include complete characterization of their contents utilizing mass spectrophotometric analysis.

165

Estrogen and Tamoxifen Modulate the Expression of Key Estrogen Metabolizing Enzymes in Endometrial Cancer Cells. Sana M Salih, Manubai Nagamani,[*] Ayman Al-Hendy,[*] Salama A Salama. *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Background: Prolonged exposure to unopposed estrogen or tamoxifen is a well-recognized risk factor for endometrial cancer. The exact mechanisms by which estrogen and tamoxifen induce endometrial carcinogenesis are not fully understood.

Estrogen is metabolized by Cytochrome p450 (1A1 and 1B1) to 2 and 4-hydroxy catechol estrogen respectively. 2-hydroxy catechol estrogen is an anti-carcinogenic metabolite while 4-hydroxy catechol estrogen is involved in mutagenesis and cancer initiation. Among the postulated mechanisms for estrogen induced carcinogenicity is cytochrome P450-mediated metabolism of estrogen into carcinogenic 4-OH catechol estrogens. These genotoxic catechol estrogen metabolites are inactivated mainly in the extrahepatic tissues by Catechol-O-Methyl Transferase (COMT). Thus, intricate balance in the expression of estrogen metabolizing enzymes in endometrial tissues is fundamental for the development of endometrial cancer.

Objectives: To investigate the effects of estrogen and tamoxifen on the expression of estrogen-metabolizing genes (CYP1A1, CYP1B1, and COMT) in endometrial cancer cells

Methods: The effect of estrogen and tamoxifen on the expression of CYP1A1, CYP1B1, and COMT genes in Ishikawa cells were assessed using western blot analysis, Luciferase reporter gene assay, and quantitative real time RT-PCR.

Results: Estrogen and tamoxifen down-regulated CYP1A1 and COMT genes expression in a concentration-dependent fashion. On the other hand, the expression of CYP1B1 was induced by estrogen and tamoxifen.

Conclusion: Our results suggested that down-regulation of CYP1A1 and COMT in concomitant with up-regulation of CYP1B1 may represent a novel mechanism by which estrogen and tamoxifen induce endometrial cancer.

166

Characterization of the Roles of Activin-A in the Regulation of Myometrium. Pasquapina Ciarmela, Ezra Wiater, Wylie Vale. (SPON: Felice Petraglia). *Peptide Biology Laboratories, The Salk Institute for Biological Studies, La Jolla, CA, USA.*

Objective: Activin-A is a growth factor with endocrine and autocrine/pacrine actions in the control and maintenance of normal reproductive function. Direct actions of Activin-A in various uterine tissue both during and outside the pregnancy are widely recognized. However, possible roles of Activin-A in the myometrium have not been investigated. In this study, we characterize if myometrial cell lines are responses to Activin-A and investigate possible roles of Activin-A in regulating myometrial function.

Methods and Results: PHM1 cells, a cell line derived from pregnant human myometrium, have previously been used as a model for myometrial cells, since they maintain smooth muscle phenotype and responses to oxytocin. In order to characterize activin responses in these cells, we first investigated expression of components of the activin signaling system. We demonstrate, as measured by RT-PCR, that these cells express the activin-bA subunit, the activin receptors ALK4, ActRII and ActRIIB, and the activin binding protein follistatin, signifying that these cells express components required for activin-A production and its signal transduction. In functional experiment, activin-A induced phosphorylation of smad-2 in PHM1 cells, as well as in hTERT HM and ELT3 cells (two other myometrial derived cell lines), as well as in rat uterus explants. We found that activin-A reduces PHM1 cell growth, in a time and dose dependant manner, and that this action can be blocked by follistatin. In order to test the role of activin-A in myometrial function we examined its effect on expression of some genes involved in myometrial function. Activin-A treatment lead to decreased expression of oxytocin receptor and HOXA 10 mRNA, but it did not alter expression of progesterone receptor A and Cox-2 mRNA levels. Furthermore, treatment of PHM1 cells with activin-A attenuate oxytocin induced intracellular Ca2+ accumulation, suggesting a possible role of activin-A in regulating myometrial cell function.

Conclusion: We have demonstrated that myometrial cells express activin receptors and signaling mediators and that activin-A can induce myometrial cell responses, regulation of cellular proliferation, and other functional responses. Activin-A inhibition of myometrial cell proliferation, and attenuation of oxytocin action, may suggest that activin-A has roles in regulating myometrial proliferation, myometrial differentiation, and uterine quiescence.

167

Adenomyosis: Incidence, Co-Incidence, and Risk Factors – A Preliminary Report. Radwan Asaad, Soubhi Zitouni, Waseem Khoder, Gene McNeeley, Elizabeth Puscheck, Michael P Diamond.* *OB/GYN, Wayne State Unvirsity (WSU), Detroit, MI, USA.*

Objectives: Study the correlation between adenomyosis and potential risk factors, as well as to identify the accuracy of ultrasound (U/S) in its diagnosis.

Methods: Medical records were reviewed for patients who underwent hysterectomy for benign indications at WSU hospitals from 1/1/05 – 4/30/06. Pathology reports were reviewed to identify the incidence of adenomyosis and the co-incidence of fibroids, endometriosis, and polyps. Patients with adenomyosis were used as a study group with the remainder as a control. Surgical history was reviewed and the potential risk factors (dilation and curettage (D&C), cesarean, myomectomy, induced abortion, hysteroscopy, and endometrial ablation) were tested in the study group versus the control using the Fisher exact test. Patients who had pre-operative ultrasound were identified and the accuracy of the test was examined against the pathologic diagnosis.

Results: 410 patients were included. Adenomyosis was confirmed by pathology in 197 patients, an incidence of 48%. Adenomyosis was the only diagnosis in 14% of the patients. The co-incidence of other pathologies was 85% for fibroids, 6% for endometriosis, and 4% for polyps. Incidence was 47% in African-Americans versus 51% in all other patients (NS). Of the 283 patients who had pre-operative ultrasound, 12 were positive for adenomyosis yielding a sensitivity of 8%, specificity of 99%, positive predictive value of 83%, and negative predictive value of 56%. Table 1 shows the odds ratios for the potential risk factors.

Conclusion: Adenomyosis was identified in about half of hysterectomy specimens, with no difference in racial incidence. D&C and induced abortion had a statistically significant correlation with the incidence of adenomyosis. Ultrasound was highly specific but not sensitive for the diagnosis of adenomyosis.

Table 1: Risk Factors

Risk Factor	Frequency in adenomyosis	Frequency in control	Odds Ratio	95% CI	P Value
D&C	100 (51%)	88 (41%)	1.49	1.10-2.20	0.048
Induced Abortion	48 (25%)	35 (16%)	1.66	1.02-2.70	0.049
Cesarean	56 (29%)	50 (23%)	1.31	0.84-2.04	0.26
Hysteroscopy	63 (32%)	54 (25%)	1.40	0.91-2.16	0.127
Myomectomy	19 (10%)	15 (7%)	1.42	0.70-2.89	0.37
Endometrial Ablation	13 (7%)	9 (4%)	1.62	0.68-3.87	0.381

168

Upregulation of MUC4 in Cervical Squamous Cell Carcinoma: Pathological Significance. Elizabeth G Munro,[1] Neel Kamal,[2] Esther Oliva,[3] Maneesh Jain,[2] Subodh M Lele,[4] Maureen P Lynch,[1] Langkai Guo,[1] Kai Fu,[4] Poonam Sharma,[5] Steve Remmenga,[6] John S Davis,[6] Bo R Rueda,*[1] Surinder K Batra.[2] *[1]Vincent Ctr. for Repro. Bio, Mass. Gen. Hosp, Boston, MA; [2]Dept. of Biochem. & Mol. Bio, Univ. of Nebraska Med. Ctr, Omaha, NE; [3]Dept. of Pathology, Mass. Gen. Hosp, Boston, MA; [4]Dept. of Pathology, Univ. of Nebraska Med. Ctr, Omaha, NE; [5]Dept. of Pathology, Creighton Univ. Med. Ctr, Omaha, NE; [6]Dept. of OB/GYN, Eppley Cancer Inst, Univ. of Nebraska Med. Ctr, Omaha, NE.*

Background: MUC4 is a transmembrane glycoprotein with upregulated expression in various cancers. Moreover, there is evidence that MUC4 expression is increased in cervical squamous dysplasia.

Objectives: Our objectives were to evaluate MUC4 expression in squamous epithelium and endocervical glands, and in situ and invasive squamous cell carcinoma (SCC) and adenocarcinoma (AC) of the cervix.

Methods: With IRB approval, 49 patients with SCC and AC (in situ and invasive) of the cervix were identified retrospectively. Tissue sections were cut from paraffin blocks, and immunohistochemistry was done using a purified mouse anti-MUC4 MAb. Semiquantitative analysis was performed by scoring intensity (0: negative, 1: weak, 2: moderate, and 3: strong) and distribution (focal <10%, multifocal = 10-60%, diffuse ≥ 60%).

Results: Glycogenated squamous epithelium (n = 16) stained for MUC4 with an average intensity of 1.09 in a patchy, basal distribution, and squamous metaplasia (n=5) stained diffusely and strongly with an average intensity of 2.80 ($p = 0.0004$). SCC in situ (n=20) stained for MUC4 with an average intensity of 2.90, significantly higher than benign epithelium ($p<0.0001$), 18 diffusely while 2 only focally. Invasive SCC (n=17) stained significantly stronger than benign epithelium with an average intensity of 2.42 ($p<0.0001$), being diffuse in 10, multifocal in 5, and focal in the other 2. The average MUC4 intensity of endocervical glands (n=25) was 2.08 with apical and luminal distribution. AC in situ (n=11) and AC invasive (n=8) were not significantly different from benign endocervical glands ($p=0.2024$, $p=0.5721$, respectively) with average intensities of 2.45 and 1.88.

Conclusions: Upregulation of MUC4 indicates a role in the development SCC of the cervix. Manipulation of MUC4 expression could lead to strategies for prevention and treatment of cervical cancer.

169

The MFMU Cesarean Registry: Perioperative Prophylactic Antibiotics at Time of Non-Laboring Cesarean Section. Mara J Dinsmoor. (SPON: Catherine Y Spong). *For the NICHD MFMU Network, Bethesda, MD, USA.*

Objective: Current recommendations favoring perioperative antibiotic prophylaxis (PROPH) for non-laboring cesarean section (NLCS) are based primarily on meta analyses. Our goal was to evaluate the efficacy of PROPH at time of NLCS in reducing the incidence of postpartum infectious complications in a large and diverse population.

Methods: We performed a secondary analysis of a prospective observational study which contains detailed data on over 39,000 cesarean sections performed at 13 clinical centers from 1999-2000. Patients were included if they had a primary or repeat NLCS performed at term, did not have an intrapartum infection (eg. chorioamnionitis, pyelonephritis or pneumonia) and were not given antibiotics at delivery for reasons other than PROPH. PROPH administration and the specific antibiotics used were at the discretion of the delivering physician. The occurrence of postpartum endometritis (PPE), wound infection (WNDINF) and other less common infection-related complications, including maternal sepsis, wound dehiscence or evisceration, necrotizing fasciitis, pelvic abscess and septic pelvic thrombophlebitis (OTHER) was compared between those who did and did not receive PROPH. Results were adjusted for smoking, payor status, gestational age and BMI at delivery, race, diabetes, antepartum infections, operative time, NLCS type (primary or repeat), and center.

Results: 9432 subjects met study criteria. The 6006 (64%) mothers who received PROPH were younger (28.9 ± 5.8 vs 30.4 ± 5.7 years; $P < 0.001$), and heavier (BMI 33.9 ± 7.3 vs 32.8 ± 6.8 kg/m^2; $P < 0.001$). Patients with PROPH were also more likely to be black (24.9% vs 18.7%; $P <0.001$), to receive public insurance (60.6% vs 39.1%; $P < 0.001$), and to have diabetes (10.5% vs 9.1%; $P=.03$) or an antepartum infection (26.4% vs 20.7%; $P < 0.001$). Relationships between PROPH and infection-related outcomes are shown in Table 1.

Table 1. PROPH and infection-related outcomes

N (%)	PROPH N=6006	No PROPH N=3426	OR (95% CI)	Adj OR (95%CI)
PPE	122 (2.0)	88 (2.6)	0.79 (0.60-1.04)	0.50 (0.36-0.71)
WNDINF	31 (0.52)	33 (0.96)	0.53 (0.33-0.87)	0.47 (0.27-0.81)
OTHER	9 (0.15)	11 (0.32)	0.47 (0.19-1.13)	0.33 (0.12-0.93)

Conclusion: The use of PROPH at time of NLCS significantly reduces the risks of PPE, WNDINF, and other less common but potentially serious infectious complications.

170

Monosialoganglioside G_{M1} Is a Putative Receptor for Group B Streptococci in Human Endometrial Cells. Pawel Goluszko,[1] Amanda Jones,[2] Chandrasekhar Yallampalli.*[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Pediatrics, University of Washington, Seattle, WA, USA.*

BACKGROUND: *Streptococcus agalactiae* (Group B Streptococcus; GBS) is associated with the asymptomatic colonization of the genital tract in 20-30% women. Up to 70% of infants born to these women will become colonized and develop early onset or late onset GBS infections. The capacity to adhere, and to invade to epithelial cells allow GBS to breech host cellular barriers. The GBS adherence to epithelium involves the high-affinity interaction with intermediary molecules of the host extracellular matrix proteins such as fibrinogen, fibronectin or laminin. We report that GBS are capable to interact directly with monosialogangioside G_{M1} expressed on the surface of human endometrial cells to promote adherence and invasion.

METHODS: GBS clinical strain A909 and its capsule-deficient and penicillin-binding protein 1a-deficient mutants were used throughout the study. Immunofluorescence microscopy and antibiotic protection assay were employed to assess the adherence and invasion of GBS to Ishikawa and

HEC-1-A cells derived from human endometrium. Monosialoganlioside G_{M1} expression on endometrial cells was tested by labeling with fluorescent conjugate of cholera toxin B subunit (CTB) and with western blot analysis. G_M1-ELISA was designed to assess whether GBS demonstrate reactivity with purified G_{M1}.

RESULTS: GBS adhered and invaded into Ishikawa cells, but failed to attach and invade HEC-1-A endometrial cells. Cholesterol depletion of Ishikawa cells did not affect bacterial binding but resulted in almost complete loss of invasiveness. Labeling endometrial cells with fluorescent conjugates of CTB subunit, which specifically binds to monosialoganglioside G_{M1}, showed that G_{M1} is expressed on Ishikawa cells but not on HEC-I-A cells. The difference in G_{M1} expression was confirmed in Western blot. In G_{M1}-ELISA GBS appeared to bind to purified G_{M1} and showed significantly lower reactivity with bovine serum albumin used as a negative control.

CONCLUSIONS: Our results suggest that monosialoganglioside G_{M1} is a cellular receptor for GBS involved in bacterial binding and invasion. Gangliosides are sialic acid-containing glycosphingolipids found on the surface of plasma membrane of vertebrate cells. Further studies are required to assess whether the expression of GM_1in female genital tract contributes to the colonization and invasive infections due to GBS.

171

Identification and Optimization of Topical Antiretrovirals: Lessons from Ciclopirox, the Vaginal Fungicide. Hartmut M Hanauske-Abel,[1] Deepti Saxena,[1] Darlene D'Alliessi Gandolfi,[2] Mainul Hoque,[1] Michael B Mathews,[1] Tsafi Pe'ery,[1] Myung Hee Park,[3] Edith C Wolff,[3] Debra S Heller,[*1] Bernadette M Cracchiolo,[1] Paul Palumbo.[1] (SPON: Laura T Goldsmith). *[1]Obstetrics, Gynecology, and Women's Health; Pediatrics; Biochemistry and Molecular Biology; Pathology and Laboratory Medicine, New Jersey Medical School-UMDNJ, Newark, NJ, USA; [2]Department of Chemistry, Manhattanville College, Purchase, NY, USA; [3]Oral and Pharyngeal Cancer Branch, National Institute for Dental and Craniofacial Research – NIH, Bethesda, MD, USA.*

Background: Greater than 90% of new infections with human immunodeficiency virus type 1 (HIV-1) are due to sexual transmission. Since a protective vaccine against HIV-1 remains elusive, topical antiretrovirals ('microbicides') are needed to reduce the number of new infections, worldwide the foremost threat to women's health. We recently demonstrated that ciclopirox blocks HIV-1 infection in culture at 1/1000 its commercial concentration, and identified as its target the crucial hypusine residue of the eukaryotic translation initiation factor 5A (eIF5A), a cellular factor recruited by HIV-1.

Objective: We hypothesized that **i**) a drug chemically unrelated to ciclopirox yet likewise suppressive of hypusine formation, displays a ciclopirox-like antiretroviral profile; and if identified, then **ii**) the antiretroviral structure-activity relation established for ciclopirox, should be applicable to guide the synthesis of derivatives with increased antiretroviral activity. We tested whether the drug deferiprone, used to alleviate transfusional iron overload, meets both criteria.

Results: In multiple experiments, deferiprone suppressed hypusine formation in vitro (IC50=60 μM) and in cells (IC90=100 μM). Acute infection of freshly isolated lymphocytes by HIV-1 isolates was dose-dependent, with complete suppression of p24 synthesis and viral copy number at 200 μM. Like ciclopirox, deferiprone triggered apoptotic ablation preferentially of infected lymphocytes. In HIV-1 infected H9 cells, a deferiprone analog carrying a cyclohexyl moiety isopositoned to the one essential for ciclopirox activity, decreased the IC50 by a factor of 10. The genomic structure of HIV-1 determined the antiretroviral activity of both drugs.

Conclusion: We propose that topical deferiprone be ranked among candidate microbicides. Its antiretroviral activity can be optimized with a ciclopirox-like hydrophobic anchor.

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172

Surgical Site Infection after Hysterectomy: Cultured Pathogens Are Resistant to Prophylactic Antibiotics. Adi Reches,[1] Yehuda Carmeli,[2] Joseph Lessing,[*1] David Pausner,[1] Anat Yerushalmi,[1] Dan Grisaru.[1] *[1]ObGyn, Sourasky Medical Center, Tel Aviv, Israel; [2]Infections Diseases, Sourasky Medical Center, Tel Aviv, Israel.*

BACKGROUND: Surgical site infection following abdominal hysterectomy has an incidence of 6-12%, despite antibiotic prophylaxis. We hypothesized that in an era of increasing antibiotic resistance, organisms causing these infections are resistant to the prophylactic antibiotic used.

METHODS: The study population included all patients who had an abdominal hysterectomy from Dec 2002 to Jan 2006, and developed surgical site infection proven by a positive culture. Prophylaxis treatment is given 30 min. prior surgery (Cefonicid 1gr); no additional antibiotics are given following surgery. In suspected surgical site infections, empirical treatment includes a combination of ampicillin, gentamicin and metronidazole. The microorganism isolated and their antibiotic susceptibility were recorded. Demographic information, medical background, operation notes and postoperative course were collected from the patients' files

RESULTS: During the study period, 620 women underwent a hysterectomy. 68 (10.96%) had a positive wound culture taken within a month. The average age was 56.7 (range 28-90) years. Surgical indications: fibroids (54.5%), endometrial ca. (32.35%), cervical ca. (7.35%), others (5.8%). TAH+BSO was preformed in 52 patients (76.5%), TAH in 13 (19.2%), STAH 1 (1.4%), radical hysterectomy 2 (2.9%). Average hospitalization after surgery was 9.3 days.

135 microorganisms were isolated: E. coli (27), Enter. faecalis (15), Staph. aureus (15) and Pseud. aeruginosa (7). 44 were found to be resistant to the prophylactic treatment and 15 were resistant to the empirical treatment.

Pseudomonal infection (not covered by prophylactic or empiric therapy) was associated with other systemic illnesses ($p < 0.008$), lung diseases ($p < 0.007$), prolonged hospitalization ($p < 0.04$) and additional surgical procedures ($p < 0.04$).

The parameters associated with gram (-) infections (resistant to the prophylactic antibiotic) include: other systemic illnesses ($p < 0.002$), hypertension ($p < 0.001$) and hypothyroidism ($p < 0.01$).

CONCLUSION: A significant portion of pathogens causing surgical site infection following hysterectomy are resistant to the prophylactic treatment, and some are resistant to the empirical treatment. There are parameters associated with their development. Changing the prophylactic and empirical treatment should be considered in high risk patients.

173

Nitric Oxide Inhibits CD55 Expression in Endometrial Cells by Interacting with No-Responsive Elements in CD55 Promoter. Pawel Goluszko, Petri Urvil, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

BACKGROUND. Decay-accelerating factor (CD55), a glycosylphosphatidyl-inositol-anchored membrane protein, is ubiquitously expressed in mammalian cells and plays a role as a regulator against the lytic action by serum complement. The CD55 expression is regulated by a variety of mechanisms including nitric oxide (NO) and Phosphoinositide 3-kinases (PI3Ks), but the mechanismsof regulation are unknown. In Ishikawa cells derived from endometrial carcinoma endogenous NO significantly decreased CD55 protein and mRNA. P13Ks regulate cell proliferation, survival, growth and motility and were reported to downregulate CD55 expression in endothelial cells. We hypothesized that NO downregulates CD55 either directly by interacting with NO-responsive elements, binding sites for Sp1 transcription factor, in the promoter region of CD55 or indirectly by inhibiting ceramide, a negative regulator of PI3Ks activity.

METHODS. The upstream region of human CD55 promoter from -794 to + 1 between the *Sac I* and *Bgl II* sites was cloned to the pCAT3 Basic vector which harbors a chloramphenicol acetylase (CAT) reporter gene. The new construct named fCD55 was transfected to endometrial Ishikawa cells. The CAT reporter gene assay was used to assess the effect of exogenously delivered NO on the transcription in CD55 promoter region. The effect of wortmannin, a PI3Ks inhibitor, on CD55 expression and transcription was assessed by CAT assay and by immunoblotting. The cellular distribution of ceramide in cells treated with NO donor was evaluated by fluorescence microscopy.

RESULTS. CAT assay revealed a significant reduction of CD55 transcription in Ishikawa endometrial cells transfected with a plasmid fCD55 and treated with NO donor. The mutation in the proximal Sp1 binding site in CD55 promoter caused inhibition of CD55 transcription. Treatment of Ishikawa endometrial cells with wortmannin increased the transcription of CD55 and the level of CD55 protein. This increase was reversed in the presence of NO donor. Treatment of Ishikawa cells with exogenous NO led to the redistribution of ceramide in these cells.

CONCLUSIONS. We demonstrated the presence of NO-responsive element(s) in the cloned promoter region of CD55. NO most likely interferes with Sp1 binding sites in the proximal site of CD55 promoter. NO may also sustain PI3Ks activity required for CD55 downregulation.

174

TLR-4, IL-1RI and TNF-R1 Are Differentially Distributed in Gestational Tissue and Fetal Brain: Implications for Unique Inflammatory Responses. Dave A Gayle, Theresa A John, Mina Desai, Ederlen Casillas, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective**:** Maternal and intraamniotic infections often lead to an inflammatory response in the fetal environment. Although cytokine induction is intrinsic to the inflammatory response, fetal cytokine responses may be associated with adverse consequences (eg, neural injury). To explore mechanisms of cytokine action during fetal development, we sought to determine the mRNA distribution of inflammatory signaling mechanisms, specifically toll-like receptor 4 (TLR4), interleukin-1 type 1 receptor (IL-RI) and tumor necrosis factor receptor type 1 (TNF-RI) in rat gestational tissue and fetal brain under basal and inflammatory conditions.

Methods**:** Samples of e18 rat chorioamnion, placenta, and fetal whole brain were homogenized in Tri-reagent and RNA was isolated according to the manufacturer's protocol. RNA was used as template in single-step RT-PCR assays using Applied Biosystems (ABS) Sequence Detection System 7000. Primer Express software (ABS) was used to design specific rat primers for IL-1RI, TLR4, TNF-RI and β-actin (which was used to normalized threshold cycle (C_T) values).

Results**:** Real-time RT-PCR assays showed a differential mRNA expression profile of IL-1RI, TLR-4 and TNF-R1 in fetal brain, chorioamnion, and placental tissues. IL-1RI mRNA was heavily expressed in the fetal brain (13.4±0.3, mean±SEM of delta C_T values) and the chorioamnion (17.8±0.4); however placental IL-1RI levels were extremely low (27.1±1.0). TLR-4 mRNA was most strongly expressed in the placenta (13.5±0.3) followed by the chorioamnion (15.4±0.3) and the fetal brain (15.5±0.3). TNF-RI mRNA was very strongly expressed in the fetal brain (8.4±0.4), whereas more modest levels were observed in the placenta (12.9±0.4) and chorioamnion (13.1±0.5). Comparisons within a signal showed that *in vitro* treatment of tissues with LPS, IL-1β, or TNF-α did not result in any significant change in any of the three receptor mRNA levels.

Conclusions: The differential expression of signaling components for LPS, IL-1β and TNF-α in the fetal brain, chorioamnion and placenta suggests unique tissue-specific responses to inflammatory stimuli. As varying bacterial types and sites of infection may have markedly different impact on the fetal environment, unique anti-inflammatory interventions may minimize adverse outcomes for the mother and fetus.

175

Fetal Brain and Gestational Tissue Cytokine Responses to Inflammatory Stimuli: A Mechanism of Neural Injury. Dave A Gayle, Theresa A John, Mina Desai, Ederlen Casillas, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Chorioamniotic infections are common during pregnancy and have been associated with adverse neurological outcomes including periventricular leukomalacia and cerebral palsy. In addition to intraamniotic infection, maternal infections induce inflammatory responses in gestational tissues and the fetus, though it is unclear how maternal inflammatory stimuli induce fetal brain responses. We sought to determine whether fetal brain and gestational tissue inflammatory responses are driven by an infectious agent or by secondary cytokine-induced responses.

Methods: We used an *in vitro* rat model to determine tissue specific production of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in response to LPS as well as select cytokines. Explant cultures of e18 fetal brain, placenta and chorioamnion were treated with LPS (50 μg/ml), IL-1β (5 μg/ml) or TNF-α (5 μg/ml) and media and tissue were collected at 6 h post-treatment to assay for cytokine production.

Results: LPS significantly ($p<0.01$) triggered fetal brain production of IL-1β (15±5 to 138±20 pg/ml) IL-6 (227±30 to 1742±234 pg/ml) and TNF-α (17±5 to 258±19 pg/ml). IL-1β also increased brain IL-6 (862±100 pg/ml) but not TNF-α. TNF-α increased brain IL-6 though not IL-1β. LPS also induced placental IL-6 and TNF-α while TNF-α induced IL-6 only. Chorioamnion explants produced IL-6 to all three stimuli.

Conclusions: Fetal brain demonstrates cytokine secretion in response to inflammatory stimuli (including cytokines), suggesting the induction of an endogenous neuroinflammatory cascade and potential neural injury. As placental and chorioamnion tissues selectively secrete cytokines which may access the brain parenchyma, optimal treatment of infections during pregnancy may include suppression of cytokine induction in addition to antibacterial agents.

176

Placental Localization and Expression of TGF-b1, TGF-bRI and p-Smad2 in Pregnancies Complicated by Preeclampsia. Tullia Todros,*[1] Elena Vasario,[1] Simona Cardaropoli,[1] Ettore Piccoli,[1] Daniela Marzioni,[2] Roberta Romagnoli,[3] Luana Paulesu,[3] Mario Castellucci.[2] *[1]Department of Obstetrics and Gynecology, University of Turin, Turin, Italy; [2]Institute of Normal Human Morphology, Polytechnic University of Marche, Ancona, Italy; [3]Department of Physiology, University of Siena, Siena, Italy.*

INTRODUCTION. Preeclampsia is characterized by an exaggerated inflammatory response. Transforming Growth Factor-b1 (TGF-b1) plays a major role in trophoblast growth and differentiation and it is also involved in the modulation of systemic inflammatory response.

OBJECTIVE. To investigate the placental localization and expression of TGF-b1, its receptor TGF-bRI and its activator phosho-Smad2 (p-Smad2) in pregnancies complicated by preeclampsia and fetal growth restriction (PE-FGR).

MATERIALS AND METHODS. We studied 17 placentas from pregnancies complicated by PE-FGR and 8 placentas from normal pregnancies. All placentas were analysed with immunohistochemistry, RT-PCR, ELISA and Western Blotting.

RESULTS. Maternal age, parity and gestational age at delivery were similar in the two groups; neonatal weight and Z-score were significantly lower in the PE-FGR group.

Immunohistochemistry showed that TGF-b1 in normal and pathological tissues was localized in the villous trophoblast, mainly in the syncytiotrophoblast, but with a more intense immunostaining in PE-FGR placentas. TGF-b1 concentrations (ELISA) were significantly higher (median 0.097 ng/ml) in PE-FGR than in normal placentas (median 0.0465 ng/ml). Immunohistochemistry showed that TGF-bRI was mainly localized in the myofibroblasts around the villous fetal vessels in normal and pathological placentas and WB analysis showed no differences in protein expression between normal and pathological tissues. RT-PCR for TGF-b1 and TGF-bRI confirmed the placental mRNA synthesis of these molecules. Immunohistochemistry for p-Smad2 in normal placentas showed a nuclear staining in the myofibroblasts around the fetal vessels and in the stroma of the placental villi; in pathological placentas tissues p-Smad2 nuclear staining was present also in the trophoblast. The percentage of the nuclei stained for p-Smad2 in the PE-FGR placentas was higher (61,5%) than in the normal placentas (38%) but the difference was not statistically significant.

CONCLUSION. The increase of TGF-b1 observed in PE-FGR placentas could be explained as an attempt to limit the exaggerated inflammatory response of PE.

177

Intrinsic Renal Vascular Adaptation to Pregnancy Is Minor. Joris van Drongelen,[1] Marc EA Spaanderman,[1] Paul ABM Smits,[2] Frederik K Lotgering.*[1] *[1]Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands; [2]Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands.*

OBJECTIVES:

The hemodynamic changes of pregnancy may represent an intrinsic vascular, humoral or autonomic adjustment. The aim of the study was to determine to what extent the renal and mesenteric arterial vasodilatation in pregnancy is due to intrinsic adaptation.

METHODS:

In non-pregnant (NP) and 11 days' pregnant (P) Wistar Hannover rats, we studied the renal and mesenteric arterial response to phenylephrine (PE) and nitric oxide (NO) blockade by L-NAME. In the Isolated Perfused Rat Kidney (IPRK) the response of the renal perfusion flow (RPF) to PE ($10^{-6.5}$ and $10^{-6.0}$ M) and L-NAME (10^{-4} M) was measured at constant pressure (90 mmHg). The myogenic response of the mesenteric arteries to PE (10^{-7} to 10^{-4} M) and L-NAME (10^{-4} M) was assessed in a wire myograph.

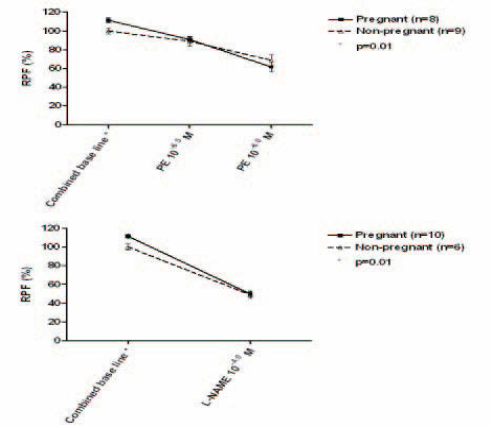
RESULTS:

Pregnancy elicits a 11 % increase in RPF ($p=0.01$). The renal response to increasing doses of PE did not differ between NP and P. In both NP and P, RPF decreased in the same extent by infusion of L-NAME at comparable rate (Figure 1).

In contrast, in mesenteric arteries the maximal response to PE in P is blunted [22 %] compared to NP ($p<0.01$), even though the sensitivity to PE is not altered. The diminished responsiveness in P can partially [12 %] be blocked by L-NAME ($p=0.01$).

CONCLUSION:
In the kidney depleted from innervation and humoral environment, the pregnancy-induced increase in RPF is 11% (in contrast to 40 % in vivo), while the response to vasoactive agents at low dose is comparable. This suggests that the intrinsic renal adaptation to pregnancy is minor, in contrast to the more profound changes in mesenteric arteries. The difference in response between vessel types is partly NO mediated.

Figure 1: Renal perfusion flow in pregnant versus non-pregnant rats. Data are presented as percentages of the non-pregnant base line value (mean ± SEM).



178

Upregulation of Renin in Cortical Connecting Tubules in Pregnant Mice, Rats, and Women. Jutta Deininger,[1] Lori Woods,[2] John Higgins,[3] Terry Morgan.*[4] *[1]Pathology, Veterans Hospital, Portland; [2]Internal Medicine, Division of Nephrology, OHSU; [3]Pathology, Stanford University Medical Center, Stanford, CA; [4]Pathology, Oregon Health & Science University, Portland, OR, USA.*

Objective: The intra-renal renin-angiotensin system is upregulated at mid-gestation in the mouse. Angiotensinogen expression is increased in proximal tubule cells and renin expression is upregulated in principal cells of the cortical connecting tubules (CNT cells in the distal nephron). It is not upregulated in pregnant transgenic mice that fail to maintain hypervolemia of pregnancy after mid-gestation. Given the apparent significance of CNT renin in renal function during pregnancy, our objective was to determine whether pregnant rats and humans also upregulate CNT renin.

Methods: We collected kidney samples from Sprague-Dawley rats before pregnancy, at mid-gestation (day 13), and term. Sections were immunostained for renin using a commercially available anti-mouse/rat renin antibody (RDI-Fitzgerald). The juxta-glomerular apparatus (JGA) served as an internal tissue control. Virgin and pregnant mouse kidneys (C57BL/6 at days 5 and 10) served as CNT renin specificity controls. Immunohistochemical studies in human kidney were performed using archival paraffin tissue blocks from autopsy specimens (collected at Stanford University Medical Center 1990-2005). Samples from non-pregnant (n=4) and pregnant (n=4, gestational age 35 weeks' ± 1 week) women were stained with a polyclonal anti-human renin antibody (kindly provided by Dr. Inagami, Vanderbilt).

Results: Both antibodies showed strong JGA immunostaining as expected. Mouse kidney sections demonstrated CNT renin staining as early as day 5. Staining in rat kidney sections was less pronounced that that observed in pregnant mice. However, weak CNT renin staining was seen at mid-gestation (day 13). Similarly, compared to non-pregnant controls, kidney samples from pregnant women show positive staining for CNT renin.

Conclusion: Our preliminary data suggest that CNT renin is upregulated in pregnant rats and women, similar to mice. Additional studies, including *in situ* hybridization and/or micro-dissection and reverse transcription-PCR of fresh frozen tissue samples will be required to confirm this observation.

179

Renal Histidine Decarboxylase Expression in the Human Fetus, Pregnant Mice, and Rats. Elizabeth DuPriest,[1] Lori Woods,[1] Susan Bagby,[1] Terry Morgan.*[2] *[1]Internal Medicine, Division of Nephrology; [2]Pathology, Oregon Health & Science University, Portland, OR, USA.*

Objective: Histidine decarboxylase (HDC), the enzyme that synthesizes the vasodilator histamine, is upregulated in the renal cortex during pregnancy in mice. Immunohistochemical evidence also suggests that it is upregulated in human fetal kidney and in pregnant women. However, recent studies show that commonly employed antibodies against HDC also cross-react with human dopa decarboxylase (DDC), making that observation less certain. In addition, others have suggested that in contrast to mice, HDC may not be expressed in rat fetal or maternal kidney. The objective of the current study was to determine whether HDC is expressed in human and rat kidney.

Methods: We used Western blot analysis to test for specific HDC isoforms in whole kidney samples from rats and humans. Renal samples from pregnant C57BL/6 mice served as positive controls. We collected kidney and liver samples from fetal rats (Sprague-Dawley), adult virgin females, pregnant rats at mid-gestation (day 13), term, and male rats. Preliminary studies were also performed using human fetal kidney (20 weeks' gestation). Western blotting and immunohistochemistry was performed using the PROGEN antibody employed in our prior work compared to the polyclonal BioVision antibody, which reportedly does not cross-react with DDC. Human DDC served as a negative control.

Results: Western blot analysis using the BioVision antibody revealed strong HDC signal at 64kDa (active enzyme) in the mouse positive control, human fetal kidney, and rat fetal kidney. Samples from adult female rats before, during, and after pregnancy showed a band at 74 kDa (proenzyme), and weaker bands at 64 and 110 kDa (dimer). There was no cross-reactivity with the human DDC control (1 ug) and no non-specific 55 kDa band was seen. In contrast, the PROGEN antibody showed strong cross-reactivity with human DDC and revealed a 54 kDa band in all samples tested that may represent either HDC or DDC. Immunostaining rat kidney sections showed only superficial cortical proximal tubule staining that did not vary in intensity or location throughout gestation.

Conclusion: Renal HDC is expressed in the rat kidney and human fetal kidney. Unlike mice and women, however, pregnant rats do not significantly upregulate renal HDC expression. Future confirmation of renal HDC expression in pregnant women may be deduced from positive immunostaining using the specific BioVision antibody.

180

The Effect of Active Cigarette Smoke Exposure on Gestational sFLT-1 Levels in the Rat. Kenneth K Chan,* Leah Battista, Yue Lan Ren. *Perinatology, Long Beach Memorial Women's Hospital, Long Beach, CA, USA.*

Objective: Women who smoke during pregnancy have a decreased risk for developing preeclampsia. Elevated sFlt-1 levels have recently been described in women who will develop the hypertensive disease. We aim to study the effects of cigarette smoke on sFlt-1 levels in the pregnant rat.

Methods: Sprague-Dawley rats at gestational ages 12-21 days (term=day 23) were subjected daily to two hours of cigarette smoke (Study Group, N=8) or ambient air (Control Group, N=8) as administered by a smoking chamber previously validated for active smoke exposures. Levels of urinary cotinine and serum sFlt-1 were measured by ELISA at days 12 (baseline) and 21 (post exposure).

Results: Urinary cotinine levels increased after exposure in the Study cohort and reflected levels (32.6 ± 3.5 ng/ml) expected of average human daily exposures to cigarette smoke. Baseline serum sFlt-1 levels were no different between Control and Study animals (59.4 ± 13.7 vs. 58.1 ± 20.8 pg/ml, respectively); nor did smoking exposure cause any differences in late-pregnancy levels (333.8 ± 129.6 pg/ml for Controls and 372.8 ± 99.8 pg/ml for Study animals).

Conclusions: Smoking of an amount typical of human use does not alter serum sFlt-1 levels in the pregnant rat. We speculate that if smoking does reduce the rate of preeclampsia in pregnant women through modulation of sFlt-1 levels, the rat model may not reflect its true pathophysiology.

181

Exogenous Platelet-Activating Factor Up-Regulates Renal Endothelin Expression in the Pregnant Rat. Xiaowu Qu, Larry G Thaete,* Sylvia Synowiec, Mark G Neerhof.* *Obstetrics & Gynecology, Evanston Northwestern Healthcare and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.*

Background: Platelet-activating factor (PAF) infusion produces fetal growth restriction (FGR) in the rat. The mechanism for PAF-induced FGR has not been identified. Increased levels of both endothelin-1 (ET-1) and PAF have been shown to be important in the pathophysiology of another model of FGR in the rat. The kidney is known to be an important site for endogenous ET-1 synthesis. Whether PAF up-regulates the expression of ET-1 has not been determined *in vivo*.

Objective: To quantify preproET-1 expression in the kidneys of the pregnant rat in response to exogenous PAF.

Methods: Two groups of pregnant Sprague-Dawley rats (n=5 each) were implanted with venous catheters on gestational day 14 (term = 22 days) and received carbamyl-PAF (2.5 µg/kg/h) or saline IV via osmotic pumps on days 14-21. The rats were euthanized on gestational day 21; their kidneys were removed and frozen for total RNA extraction and analysis of preproET-1 mRNA expression by real-time quantitative PCR.
Results: After seven days of infusion, the ratio of preproET-1 mRNA copy number normalized to 18S rRNA was significantly higher in the kidneys of PAF-treated rats, compared with the saline treated animals. Means and standard errors for the ratio of preproET-1 mRNA/18S rRNA (X 10^3) were 0.46 ± 0.07 and 0.89 ± 0.15 for the saline and PAF treatment groups, respectively ($p<0.05$ using an unpaired student *t* test).
Conclusions: Endothelin-1 transcription is up-regulated by PAF in the kidneys of the pregnant rat. ET-1-mediated vasoconstriction may reduce uterine and placental perfusion in this model, as we have demonstrated in other models of FGR. ET-1-mediated reduced placental perfusion, then, may be the cause of the FGR observed in response to exogenous PAF.
Supported by NIH grants HD01484 and HD046968.

182

Validation of an Automated Device to an Auscultatory Standard for Use in Pregnancy – The Dinamap ProCare. Annemarie De Greeff, Andrew H Shennan. (SPON: Lucilla Poston). *Division of Reproduction and Endocrinology, King's College London, London, United Kingdom.*
BACKGROUND Automated devices are being increasingly used as an alternative to the mercury sphygmomanometer. Accuracy in one population cannot be assumed in special populations e.g. pregnancy. The algorithm of the Dinamap ProCare is modelled on an auscultatory standard and the device achieved the criteria of both the International protocol of the European Society of Hypertension and the British Hypertension Society (BHS) protocol in an adult population. The ProCare is a robust device with potential for use on labour wards. We evaluated the accuracy of this device in a pregnant population, including women with pre-eclampsia, according to the BHS protocol. METHODS Ethics approval was obtained and thirty women (including 5 with pre-eclampsia) were recruited at a large teaching hospital. Nine sequential same arm blood pressure measurements were taken by two observers, alternating between a mercury sphygmomanometer and the device. The ISSHP definition of pre-eclampsia was adhered to and the data was analysed according to the BHS guidelines. RESULTS The Dinamap ProCare achieved an A/A grade according to the BHS protocol with 77%, 91% and 99% of systolic differences and 71%, 86% and 96% of diastolic differences within 5, 10 and 15mmHg of the mercury standard. It also satisfied the criteria of the Association for the Advancement of Medical Instrumentation with a mean and standard deviation of 1.2 (5.8) mmHg and 0.1 (7.6) mmHg for systolic and diastolic pressures respectively. CONCLUSION The Dinamap ProCare is the first robust device currently available, to achieve recommendation according to the BHS criteria in a pregnant population. Further assessment is required to confirm its accuracy in pre-eclampsia.

183

Changes in Arterial Pulse Wave Parameters in Normal Human Pregnancy, and Comparison of Different Ethnic Groups. Asma A Khalil, Derek J Cooper, Kevin F Harrington. (SPON: Donald M Peebles). *Obstetrics and Gynaecology, Homerton University Hospital, London, United Kingdom.*
Objective
To establish normal ranges for arterial pulse wave parameters in pregnancy and to investigate potential ethnic variation.
Outside pregnancy, analysis of the arterial pulse wave provides valuable information in hypertension and vascular disease. It may also distinguish the vascular changes of established pre-eclampsia (PE) from normal. However, data on the normal changes in pregnancy are sparse and need to be accurately defined before the possibility of screening for PE can be explored.
Methods
The radial artery pulse waveform was recorded in 168 healthy pregnant and 20 non-pregnant women using applanation tonometry. A mathematical translation was applied to derive the aortic waveform. Augmentation pressure (AP) and Augmentation Index (AI) were calculated. The results in the two major ethnic groups in our pregnant population [Caucasian (94) and Afro-Caribbean (74)] were compared.
Results
Mean AP (3.32 versus 6.8) and mean AI (15.8 versus 24.6) were significantly lower in pregnancy than non-pregnancy (p=0.001). There was no significant difference in AP or AI across the three trimesters. There was no variation between the two ethnic groups studied.
Conclusions
Our work has defined the normal changes in arterial wave characteristics in pregnancy, confirming a fall in vascular resistance in pregnancy. These data provide the foundation for further investigation into the potential role of the technique in diagnosis and prediction of vascular disease in pregnancy, in particular PE.

184

Telemetry of Maternal Blood Pressure from Pre-Pregnancy to Term in Baboons Housed in a Social Environment. Mark J Nijland, Susan L Jenkins, Natalia E Schlabritz-Loutsevitch, Thomas J McDonald,* Peter W Nathanielsz.* *Dept Ob/Gyn, UTHSCSA, San Antonio, TX, USA.*
The baboon is a well established pregnancy model, yet little data exist regarding the maternal cardiovascular response to pregnancy in this species. Blood pressure (BP) during pregnancy in humans is collected once pregnancy is confirmed, typically starting at the first antenatal check. Telemetry provides a high resolution, stable means of measuring BP over time. The baboon provides the unique opportunity for BP and heart rate (HR) measurement before and during pregnancy in the same individual.
Pressure telemeters were implanted in 6 non-pregnant baboons (8 - 13 years old). Animals were housed within stable groups of 16 and freely exercise and socialised throughout the study. Each day animals entered a feeding system as part of another ongoing study. The animals spent an overnight in the system biweekly when telemetered recordings were made.
BP and HR were measured in from 2 weeks before pregnancy to term (180 days gestation). Four overnights provided average systolic, diastolic, mean arterial pressure (MAP) and HR every two weeks. No difference was found between MAP and HR recorded using telemetry compared to recordings using indwelling catheters from individually housed baboons matched for gestational age and time-of-day (Fig 1A). Systolic, diastolic and MAP declined after day 14 and were lower than pre-pregnant values during the first 6 weeks of the second trimester (Fig 1B).
In normotensive pregnant women systolic and diastolic blood pressures decrease from the first to the second trimester and increase again in the third, independent of parity or maternal age. We demonstrate that a similar response trend is also found in the baboon, thereby further highlighting the utility of this animal model in pregnancy research.

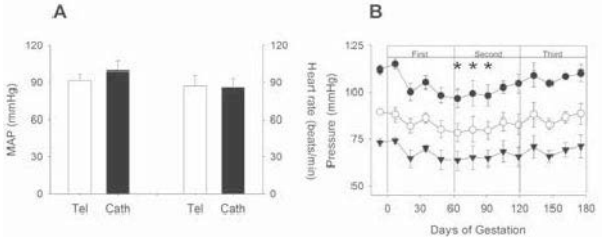


Fig 1: A Mean arterial pressure (MAP) and heart rate using telemetry in group housed animals (Tel) and indwelling catheters in animals on tether (Cath). **B.** Systolic (closed circle), MAP (open circle) and diastolic (triangle) pressures before and during pregnancy. Mean ± SEM, n=6. * $P<0.05$ ANOVA; Bonferroni tests compared to pre-pregnancy.

185

Effect of Diabetes on Endothelial Function in Uterine Arteries of Pregnant Mice. Joanna L Stanley,[1] Haiju H Chirayath,[1] Sandra T Davidge,*[2] Michael J Taggart,[1] Philip N Baker.*[1] *[1]Division of Human Development, University of Manchester, Manchester, United Kingdom; [2]Departments of Obstetrics/ Gynecology and Physiology, University of Alberta, Edmonton, AB, Canada.*
Background Pregnancy complicated by diabetes is associated with maternal and neonatal mortality and morbidity. It has previously been demonstrated that endothelial-dependent vasodilation is impaired in small myometrial arteries from women with gestational diabetes compared with normal pregnancy. Endothelial dysfunction induced in the uterine vascular bed during pregnancy would compromise blood flow to the fetus. Therefore diabetes-induced endothelial dysfunction may play a role in mediating the complications observed in diabetic pregnancies.
Aim To determine endothelial function in uterine arteries from pregnant diabetic C57Bl6/J mice.

Methods Female C57Bl6/J (18-22g) mice were injected with streptozotocin (STZ, 200 mg/kg i.p.) or vehicle (saline) 4 weeks prior to mating. Blood samples were taken, immediately before STZ administration then twice weekly for 4 weeks, and blood glucose concentrations determined. Induction of diabetes was indicated by a blood glucose concentration consistently > 10mM. Mice were culled at day 19 of pregnancy (term) and uterine arteries dissected, mounted onto a wire myograph, normalised at 0.9 of $_{L13.3kPa}$ (~55mmHg) and equilibrated (37ºC; 5%CO_2/air). Arteries were constricted with phenylephrine (PE, 10^{-5}M) and a concentration-response curve to the endothelium-dependent relaxant acetylcholine (ACh10^{-10}M-10^{-6}M) constructed.
Results Mean ± SEM blood glucose concentration was significantly higher in diabetic mice (14.78±0.7 mM) compared with vehicle (7.12±0.1 mM, $p<0.001$, t test). Mean pup weight from diabetic mice was significantly lower (0.52±0.02 vs 1.26±0.05g, $p<0.0001$, t test). Mean arterial constriction in response to PE in vehicle (active effective pressure 12.71±1.8kPa, n=7) and diabetic (12.58±3.1kPa, n=4) mice was comparable. Although similar maximal relaxation responses to ACh were attained in vehicle and diabetic mouse arteries, sensitivity was significantly reduced ($p<0.001$, t test) in the arteries from diabetic mice (EC_{50} 21.68±4.2 nM vs 6.43±2.9 nM in vehicle).
Conclusions: Endothelium-dependent relaxation was significantly attenuated in uterine arteries of diabetic mice compared with controls, suggesting diabetes does induce endothelial dysfunction in the uterine vascular bed of mice.
Supported by MFN Training grant (CIHR).

186

Immunolocalization and Pharmacological Characterization of Adrenomedullin Receptors in Pregnant Rat Mesenteric Artery. Gracious R Ross,[1] Madhu S Chauhan,[1] Uma Yallampalli,[1] Sunil Wimalawansa,[2] Chandrasekhar Yallampalli.[*1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Endocrinology, Metabolism and Nutrition, Robert Wood Johnson University Hospital, New Brunswick, NJ, USA.*
Adrenomedullin (ADM) mediates its effects through either ADM_{22-52}–sensitive ADM_1 receptors or ADM_2 receptors which can be antagonized more potently by calcitonin gene-related peptide $(CGRP)_{8-37}$ than ADM_{22-52}. Both ADM_1 and ADM_2 receptors consist of Calcitonin receptor-like receptor (CRLR), receptor activity modifying protein $(RAMP)_2$ or $RAMP_3$, respectively. We proposed to immunolocalize ADM receptor components and characterize their involvement in ADM –induced relaxation, using its antagonists, ADM_{22-52} and $CGRP_{8-37}$ in mesenteric artery (MA) from pregnant rats.
OBJECTIVE: To localize the receptor components of ADM and characterize receptor subtype involved in ADM –induced relaxation of MA from pregnant rats.
METHODS: 1) Immunohistochemistry was performed on sections of MA using anti-CRLR, -$RAMP_2$ or –$RAMP_3$ antibody; 2) Isometric tension was measured in endothelium–intact MA from day 18 pregnant rats. Relaxation responses to cumulative doses of ADM were assessed on vessels precontracted with EC_{70} of norepinephrine. After 30 min incubation with the antagonists, the concentration–response to ADM was repeated. 3) Curves were fitted to all data by non-linear regression using Prism Graph pad software to calculate EC_{50} and Schild slopes and pA_2 values were calculated.
RESULTS: Immunohistochemistry revealed almost homogenous expression of CL, $RAMP_2$ and $RAMP_3$ proteins both in endothelial and smooth muscle cells of MA. ADM concentration–dependently relaxed the endothelium–intact MA. Both ADM_{22-52} and $CGRP_{8-37}$ concentration- dependently shifted the response curve of ADM parallely to the right without affecting E_{max}. Schild plot analysis yielded pA_2 values of 4.9 for ADM_{22-52} and 7.9 for $CGRP_{8-37}$ with a slope no different from unity, 0.94 ± 0.5 (ADM_{22-52}) and 0.95 ± 0.3 ($CGRP_{8-37}$).
CONCLUSION: All components of AM_1 and AM_2 receptors are localized in endothelial and smooth muscle layer of MA. However, pharmacological characterization revealed that ADM-induced relaxation is predominantly mediated through ADM_2 receptors as the reported pA_2 values of ADM_{22-52} and $CGRP_{8-37}$ estimated in previous functional studies for ADM_1 receptors were in the order of 7 and around 6, while those for ADM_2 receptors were < 5.5 and 6.1, respectively.

187

Specific Receptor Activity Modifying Proteins (RAMPs) Are Involved in cAMP Generation by CGRP Peptides in Rat Vascular Smooth Muscle. Uma Yallampalli, Gracious R Ross, Madhu S Chauhan, Chandrasekhar Yallampalli.[*] *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
INTRODUCTION: Calcitonin gene-related peptide (CGRP) family peptides are potent vasodilators. We have shown that CGRP, adrenomedullin (ADM), and intermedin (IMD) relax mesenteric arteries and these effects are amplified with pregnancy. These family peptides utilize a common 7TM receptor, calcitonin receptor-like receptor (CRLR) and a specific RAMP. Specificity of these peptides actions are related to the type of RAMP(s) associated to CRLR. CGRP uses $RAMP_1$ and $RAMP_3$, where as ADM utilizes $RAMP_2$ and $RAMP_3$. Intermedin, however, is reported to work through all three RAMPs.
OBJECTIVES: To assess the involvement of specific RAMPs in mesenteric artery relaxation induced by each of the CGRP family peptides, CGRP, ADM and IMD, using the angonists $CGRP_{8-37}$, ADM_{22-52}, and IMD_{17-47}.
METHODS: Cyclic AMP generation in response to CGRP, ADM and IMD in the presence and absence of their antagonists was measured in isolated rat mesenteric arterial muscle cells. Cells in culture were treated with 1 µM of $CGRP_{8-37}$, ADM_{22-52} or IMD_{17-47}, for 1 h in serum free DMEM. Cells were then challenged with 100 mM CGRP, ADM or IMD for 5 min. All cells were treated with IBMX (0.1 mM) for 1 min prior to peptide treatments. Media was removed and 65% ethanol was added to the cells and sonicated to lyse cells and centrifuged. Supernatant was evaporated and cAMP was measured using cAMP RIA kit.
RESULTS: Cyclic AMP generation in rat smooth muscle cells was significantly increased by all three peptides; AM is the most potent (26-fold over controls) and IMD is the least potent (3.0-fold), whereas CGRP induced increase was 4.2-fold. Effects of CGRP are completely blocked by $CGRP_{8-37}$ and not affected by ADM_{22-52} or IMD_{17-47}. ADM effects are approximately 90% blocked by $CGRP_{8-37}$ and IMD_{17-47}, whereas, only less than 10% by AM_{22-52}. This observation is further strengthened by the abundant presence of $RAMP_3$ expression in these cells. Effects of IMD are completely blocked by $CGRP_{8-37}$ and IMD_{17-47}, whereas, only 33% by ADM_{22-52}.
CONCLUSIONS: All three CGRP family peptides stimulate cAMP in rat vascular smooth muscle cells maximal effects with ADM followed by CGRP and IMD. Inability of ADM_{22-52} to block ADM effects and the ability of $CGRP_{8-37}$ and IMD_{17-47} to block ADM effects provides evidence for $RAMP_3$ involvement in ADM induced relaxation in mesenteric artery smooth muscle cells.

188

Estrogen Modulation of Endothelial Nitric Oxide Synthase (eNOS) Phosphorylation Responses in Follicular, Luteal and Pregnant Derived Ovine Uterine Artery Endothelial Cells (UAEC). C Kevin Huls,[1,4] Dinesh Shah,[*1,4] Ronald R Magness,[*1,2,3] Gladys E Lopez.[1] *[1]Depts Ob/Gyn Perinatal Res Labs; [2]Anim Sc; [3]Peds; [4]Maternal Fetal Med, UW-Madison.*
Compared to ovine Luteal(Lut) Phase, the Follicular(Fol) Phase and Pregnancy(Preg) exhibit elevations on UBF and estrogen in association with greater NO production and eNOS expression. Phosphorylation of eNOS is a signaling marker of activation. UAEC culture models evaluate eNOS phosphorylation responses to vascular mediators. We **hypothesized** that UAECs from Fol and Preg sheep will show greater eNOS phosphorylation than Lut phase derived UAECs, and with more robust responses to ATP, VEGF, and Ionomycin(IM). Furthermore, eNOS phosphorylation will be augmented by exogenous estrogen. **Methods:** UAs obtained *ex vivo* were compared to UAECs (Passage 4-5) from Lut, Fol and Preg. UAECs were cultured in the absence or presence of E2B (10nM) for 48 hr. At 80% confluence cells were serum starved (4 hr) and subjected to: Control, ATP (100uM), IM (1uM) or VEGF (10ng/ml) for 10min. Ser635-peNOS and total eNOS were evaluated by Western. Data for Ser635-peNOS were normalized to total eNOS. **Results:** *Ex vivo* UA peNOS levels were greater in Fol and Preg UAs compared to Lut. UAEC peNOS was elevated ($P<0.05$) in Preg (1.40 $\pm$ 0.25) when compared to Lut (0.67$\pm$ 0.15) and Fol (0.35$\pm$ 0.04), which were also different ($P<0.05$). Treatment of UAECs with exogenous E2B did not alter peNOS for Fol, Lut or Preg. All UAECs responded to ATP with elevations in peNOS with a rise in Fol (2.78 fold), Lut (1.58 fold), and Preg (1.59 fold). With E2B, ATP increased Preg-UAECs (3.13 fold) peNOS response over controls, but E2B did not increase Lut or Fol UAECs. VEGF induced rises of peNOS in Fol UAECs with and without E2B (2.05 and 2.3 fold respectively). IM caused peNOS rises in Lut (2.9 fold) and Fol (3.5 fold), but not P-UAECs. This IM response was enhanced after E2B

(3.9 fold) only in Lut UAECs. **Conclusions:** *In vivo* and *in vitro* UAECs have elevated Ser635-peNOS in Preg, but this effect is lost in Fol UAEC cultures and cannot be restored with E2B. Fol UAEC peNOS responses to ATP and VEGF were greater than with Lut or Preg UAEC. When exposed to 48hr E2B, only the Preg UAECs increased their response to ATP. IM altered peNOS levels in Lut and Fol UAEC, but surprisingly did not alter peNOS levels in P-UAECs. This suggests that Ca+ is not a regulator of eNOS phosphoryation at Ser635 in Preg-UAEC. *NIH HL49210, HD38843.*

189

Shear Stress and Uterine Blood Flows during the Proliferative and Secretory Phases of the Menstrual Cycle and Early Pregnancy. Ronald R Magness,[*1,2,3] Kreg M Grindle,[1] Terrance M Phernetton,[1] David J Magness,[1] Adrienne L Schonberg,[4] Ira M Bernstein.[*4] *[1]Depts Ob/Gyn Perinatal Research Labs, UW-Madison; [2]Anim Sci; [3]Peds; [4]Dept Ob/Gyn, Univ of Vermont.*

Shear stress is a potent physiologic stimulus for endothelial NO production and induces flow-mediated vasodilation. Shear stress is directly proportional to rises in blood flow and viscosity and inversely proportional to internal radius cubed. Elevations in uterine blood flow (UBF) are seen in the menstrual cycle Secretory vs Proliferative phase and UBF increases during gestation. Our studies measured changes of *in vivo* shear stress during the menstrual cycle and at 12 weeks of gestation, testing the *hypothesis* that compared to the Proliferative and Secretory phase further UBF elevations account for increases in shear stress during early gestation. **Methods:** During the Proliferative (n=100) and Secretory phases (n=55) and at 12 weeks (n= 12) of pregnancy UA, velocity and internal radius were measured bilaterally (5 replicates/side) using high-resolution Doppler ultrasound; angle of sonoincidence < 60. Heparinized blood was collected and viscosity measured. **Results:**

(***P<0.01)	Proliferative; D=1-15 (n=100)	Secretory; D=19-26 (n=55)	Early Pregnancy 12 Weeks (n=12)
UA Internal Radius (cm)	0.096 ± 0.002	0.096 ± 0.002 NSD	0.128 ± 0.005***
UBF (ml/min)	15.0± 0.9	27 ± 2***	110 ± 13***
UAVelocity (cm/sec)	9.3 ± 0.57	15.3 ± 1.0***	32.2 ± 3.4***
Shear Stress (dynes/cm2)	20.7 ± 1.5	37.8 ± 2.62***	39.5 ± 4.8***

Compared to Proliferative phase, hematocrit and blood viscosity were decreased at 12 weeks of gestation (P<0.05). Internal radius of UA was unaltered by the menstrual cycle, but was greater at 12 weeks (+33%). Compared to Proliferative phase, Secretory phase women showed significant rises in UBF and UA Velocity that continued to rise into early pregnancy. In contrast to UBF changes and our hypothesis, Shear Stress increased equally (87%) in Secretory phase and Early Pregnant women. **Conclusions:** Equivalent rises in shear stress during the Secretory phase and 12 weeks of gestation shows an increase in radius and a profound remodeling in the 12 week UAs. Initial and maintained elevations in shear stress during the Secretory phase through early gestation will provide a profound stimulus for continued increases in NO production by UA endothelium. *NIH HL49210, HD38843, HL63101, HL71944.*

190

Uterine Artery Shear Stress in Pregnancy: Altitude, Ethnic Ancestry and Preeclampsia. Ronald R Magness,[*1] KM Grindle,[1] E Balanza,[3] L Echalar,[3] E Vargas,[3] Stacy Zamudio.[*2] *[1]Depts Ob/Gyn Perinatal Research Labs, UW-Madison; [2]Dept Ob/Gyn Women's Health, NJ Med Sch; [3]Instituto Boliviano Biologia Altura, La Paz Bolivia.*

Andean natives at high altitude have less IUGR than European migrants. Migrants showed >30% reduction in uterine artery (UA) blood flow (BF) during pregnancy, due to reduced radius, suggesting failure of shear-stress-induced flow-mediated vasodilation and remodeling. **Shear stress (SS) = 4* BF * viscosity/π r^3** whereas **Shear Rate (SR) = 4*Velocity/r**. We asked if altitude alters SS and SR and contributes to decreased BF, UA remodeling and ethnic variation in birth weight. **Methods:** Women of European vs. Andean ancestry were studied 0-10 days prior to delivery at 300 and 3600m. UA BF velocity and radius was measured bilaterally (4-5 replicates/side) using Doppler ultrasound. Viscosity was measured with a cone-plate viscometer. **Results:** UA SS and SR were reduced in Andean vs. European women regardless of altitude. Andean had significantly greater UA BF than European regardless of altitude. Decreased SS is explained by greater UA radius in Andean vs European. PE women had lower SS, SR and UA blood flow, despite only a marginal decrease in UA radius. **Conclusions:** We provide the first *in vivo* estimates of UA SS and SR in low vs. high altitude pregnancies and in PE. UA Ethnic-specific SS and SR were maintained at low vs high altitude, despite reduction in UA radius. Normalization of shear is important for UA vascular remodeling in pregnancy. Possible interpretations include ethnic set-point variation at which flow-mediated vasodilation or vascular remodeling occurs, or that impedance is generally lower in women of Andean ancestry. PE women show failure of SS-induced flow-mediated vasodilatation and UA remodeling. *NIH HL49210, HD38843, HD42737, NSF BCS 0309142.*

*p<0.01 Altitude, † p<0.01 Ethnicity, **PE

	Birth weight (g)	Viscosity (cp)	Shear Stress (dynes/cm^2)	Shear Rate (sec-1)	UA radius (cm)	UA blood flow (ml/sec)
Andean 300m (n=42)	3511±47	4.12	33±2	558±33	0.24±0.01†	6.1±0.3
Andean 3600m (n=40)	3261±69†*	4.96	31±2†	516±38†	0.23±0.01†*	4.8±0.3†*
European 300m (n=47)	3424±64	4.31	42±2	710±38	0.21±0.01	5.4±0.4
European 3600m (n=30)	2998±59*	5.12	40±3	729±38	0.20±0.01*	3.9±0.3
PE Andean 3600m (n=10)	2038±120**	5.00	22±2**	346±53**	0.21±0.02	2.9±0.5**

191

Large Conductance Ca^{2+}-Dependent K^+ Channels (BK_{Ca}) Contribute to Attenuated Uterine Vascular Responses to α-Stimulation in Pregnant Sheep. Charles Rosenfeld,[*] Timothy A Roy. *Division of Neonatal-Perinatal Medicine, UT Southwestern University at Dallas, Dallas, TX, USA.*

Background: Uterine responses to infused angiotensin II (ANG II) and α-agonists decrease in pregnant women and sheep, and responses to ANG II are mediated via α-stimulation. BK_{Ca} are expressed in uterine vascular smooth muscle (VSM) and contribute to regulation of uterine blood flow (UBF) in pregnant sheep. BK_{Ca} activity increases with rising intracellular Ca^{2+}, modifying constriction responses. This role of BK_{Ca} has not been studied in pregnancy. **Objective:** Determine if uterine VSM BK_{Ca} contribute to attenuated uterine responses to α-stimulation in pregnant sheep. **Methods:** Pregnant ewes (n=8) were instrumented with vascular catheters and uterine artery flow probes to continuously record arterial pressure (MAP), heart rate (HR), and UBF while infusing local uterine-arterial tetraethylammonium (0.005-0.04 mM), a BK_{Ca} inhibitor at <1.0 mM, and systemic phenylephrine (PE, 1.29-129 μg/min), an α_1-agonist, in Early (101-117d) and Late (135-147d) gestation. Uterine vascular resistance (UVR) was calculated. Data were analyzed by least mean squares with ANOVA. **Results:** Basal MAP and UVR fell and HR and UBF rose between 101 and 147d gestation, but only UVR changed significantly, P=0.047. PE elicited dose-dependent rises in MAP and UVR and falls in HR and UBF in both study periods (P<0.001). Except for attenuated pressor responses in Late vs. Early gestation (P=0.008), other responses to PE were similar. In Early gestation, TEA did not have a dose effect; however, uterine-arterial TEA infusions enhanced PE-induced decreases in UBF (P≤0.018) and increased pressor responses at the highest PE doses, P=0.002. Other measures were unaffected by TEA. In contrast, Late gestation was associated with dose effects of TEA on UBF and MAP, P≤0.047; again, uterine-arterial TEA enhanced PE-induced falls in UBF and rises in MAP, P=0.007. The attenuated pressor responses to PE in Late gestation persisted during TEA infusions (P=0.004), resembling that in Early gestation. **Conclusions:** We have demonstrated for the first time that uterine VSM BK_{Ca} are involved in regulating basal uterine vascular tone and UBF in ovine pregnancy as well as attenuating uterine vascular responses to α-agonists, contributing to the protective mechanisms that maintain UBF and insure fetal well-being in pregnancy. BK_{Ca} also modify pressor responses to PE, thereby contributing to attenuated systemic reactivity in pregnancy.

192

Human Uterine Arteries Express an Endothelium-Derived Contracting Factor and Large-Conductance K^+ Channels That Modulate Constrictor Responses, Basal Tone and Nitroprusside-Induced Relaxation. Charles Rosenfeld,[*] Kevin DeSpain, Xiao-tie Liu. *Division of Neonatal-Perinatal Medicine, UT Southwestern Medical Center at Dallas, Dallas, TX, USA.*

Background: The mechanisms regulating human uterine artery (UA) function remain unclear. In sheep, large-conductance calcium-dependent K^+ channels (BK_{Ca}) are expressed in UA smooth muscle and contribute to estrogen-induced vasodilation and regulation of basal uterine blood flow during pregnancy. We identified BK_{Ca} in human UA by patch clamping, but their function is unstudied. **Objective:** Examine nonpregnant human UA function and the role of BK_{Ca} in the regulation of basal tone and responses to nitric oxide (NO). **Methods:** UA were collected from 19 nonpregnant women at hysterectomy. We studied

dose responses to KCl, the α_1-agonist phenylephrine (PE), angiotensin II (ANG II) and sodium nitroprusside (SNP) in UA rings with and without endothelium and the effects of BK_{Ca} inhibition with tetraethylammonium (TEA ≤1.0 mM) on basal tone and SNP responses in denuded UA rings. BK_{Ca} expression was examined by immunoblot. Data were analyzed by 1- and 2-way ANOVA. **Results:** Dose-dependent increases in force (gm) occurred with all vasoconstrictors (P≤0.002) and were greater in endothelium-intact UA rings (P≤0.003). As in sheep, maximum ANG II responses were <25% of maximum PE responses. SNP dose-dependently relaxed UA rings precontracted with 10^{-6} M PE (P<0.001), and this was attenuated in endothelium-intact rings (P=0.002). BK_{Ca} α-subunits were detected at 83 kDa in denuded UA by immunoblot (n=4). TEA dose-dependently increased basal tone in denuded UA rings, force (gm) increasing 6- and 9-fold greater with 0.5 and 1.0 mM, respectively, vs. 0.2 mM TEA (P=0.04). TEA also dose-dependently (P=0.007) decreased SNP-induced relaxation in denuded rings, responses falling 5% and 18% with 0.5 and 1.0 mM, respectively. **Conclusions:** These are the first data showing that, as in sheep, BK_{Ca} are expressed in human UA smooth muscle and contribute to the regulation of basal tone and NO-induced relaxation, demonstrating a potential role in regulating uterine blood flow. The presence of endothelium resulted in enhanced constrictor and attenuated relaxation responses, suggesting release of an endothelium-derived constricting factor (EDCF) in human UA. Studies are underway to identify the EDCF and to characterize the molecular constituents of the human BK_{Ca}.

193

Greater Uterine Artery Blood Flow during High-Altitude Pregnancy in Indigenous (Andean) Than Foreign (European) Women. Megan J Wilson,[1] Miriam Lopez,[2] Marco Vargas,[3] Colleen Julian,[1] Wilma Tellez,[3] Armando Rodriguez,[3] Abigail Bigham,[4] J Fernando Armaza,[3] Susan Niermeyer,[1] Mark Shriver,[4] Enrique Vargas,[3] Lorna G Moore.*[1] *[1]Department of Health and Behavioral Sciences, Pediatrics/Neonatology and the Altitude Research Center, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA; [2]Clinica del Sur, La Paz, Bolivia; [3]Instituto Boliviano de Biologia de Altura, Universidad Mayor de San Andres, La Paz, Bolivia; [4]Genetics Laboratory, Department of Anthropology, Pennsylvania State University, State College, PA, USA.*

Objective: Hypoxia-associated reductions in birth weights are diminished in multigenerational compared with shorter-term residents of high altitude, but this protection is not due to differences in arterial O_2 content. We asked if uterine artery (UA) blood flow raised uteroplacental O_2 delivery to a greater extent in multigenerational (Andean) vs. shorter-term (European) residents of high altitude (3600 m). **Methods:** Serial studies were conducted using Doppler ultrasound at pregnancy weeks 20, 30, 36 as well as 3-4 mo postpartum in 42 Andean and 26 European residents of La Paz, Bolivia, confirming population ancestry with genetic markers. **Results:** Pregnancy increased UA diameter to a greater extent in the Andean than European women, thus raising UA blood flow and O_2 delivery 6-fold in the Andeans and 3-fold in the Europeans. The Andeans also had greater common iliac (CI) and external iliac (EI) flows in combination with greater UA/EI and lower EI/CI, suggesting redistribution of lower extremity flow to favor the UA in the Andean compared with European subjects. After adjusting for known covariates, fetal biometry was greater in the Andean than European babies at weeks 20 and 30, and birth weights 209 gm heavier. Lower UA resistance index (RI) correlated with larger AC at week 36 in the Andeans but not the Europeans. **Conclusions:** We concluded that Andean residents were protected from altitude-associated reduction in fetal growth by being able to maintain a normal pregnancy-associated increase in UA blood flow. Future studies are required to determine whether genetic factors are responsible and, if so, the genes involved.

194

Differentiation of Local Versus Systemic Influences on Uterine Vascular Reactivity during Pregnancy in the Single-Horn Gravid Rat. Robert R Fuller, Natalia I Gokina, George Osol.* *Department of Obstetrics and Gynecology, University of Vermont College of Medicine, Burlington, VT, USA.*

Objective. Although this functional adaptation is essential for normal pregnancy outcome, the underlying mechanisms have not been identified. The objective of this study was to differentiate local vs. systemic influences of pregnancy on vacular reactivity in the rat uterus by using a one horn ligation model in conjunction with agonists selective for smooth muscle or endothelial activation.

Study design. A total of 12 Sprague-Dawley rats underwent unilateral uterine horn ligation to create animals with single uterine horn pregnancies. Uteri were harvested on day 20 of gestation. Segments of uterine arcuate arteries were isolated and studied at 50 mm Hg intralumenal pressure using an arteriograph and videomicroscopy for recording vessel diameters. Concentration-response curves to phenylephrine, sodium nitroprusside (SNP), and acetylcholine were obtained using paired vessels from the pregnant and non-pregnant horns of the same animal. Paired t-tests were used for statistical analyses.

Results. Sensitivity to phenylephrine-induced vasoconstriction was significantly increased in the pregnant horn compared to the non-pregnant horn ($-\log(EC_{50})$ = 6.58 ± 0.10 vs 6.28 ± 0.07, p=0.03), along with a reduction in sensitivity to SNP-induced vasodilation ($-\log(EC_{50})$ = 6.27 ± 0.38 vs 7.49 ± 0.24, p=0.03). The effects observed with phenylephrine and SNP on these arteries in the pregnant and non-pregnant horns were similar to the effects observed between pregnant and non-pregnant animals. In contrast, there were no differences in acetylcholine-induced vasodilation between the uterine horns ($-\log(EC_{50})$ = 7.15 ± 0.17 vs 7.31 ± 0.14, p=0.55).

Conclusions. The phenylephrine and SNP reactivity data suggest that local factors predominate in smooth muscle adaptation during pregnancy; conversely, the acetylcholine responses argue for systemic influences being of principal importance in endothelial adaptation. Taken together, our results raise the novel possibility that uterine vascular remodeling during pregnancy may be differentially regulated by local vs systemic factors depending on cell type.

195

Local Uteroplacental Influences Are Responsible for the Induction of Rat Uterine Artery Myogenic Tone during Pregnancy. Natalia I Gokina, Robert R Fuller, George Osol.* *Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*

Introduction: Previous data have shown that uterine artery constrictor responses to elevation of intraluminal pressure (myogenic tone) are considerably enhanced in late pregnant rats. In this study, we hypothesized that factors associated with the site of placentation are critically involved in this adaptive process. The objectives were to: (1) differentiate between systemic and local influences in effecting myogenic tone by utilizing a surgical ligation model in which implantation is restricted to one of two uterine horns; (2) determine whether augmented myogenic tone is due to increased elevation in smooth muscle intracellular $[Ca^{2+}]_i$; and (3) define the role of the endothelium in differential myogenic behavior of uterine arteries from the non-gravid vs. gravid uterine horn.

Methods: Radial uterine arteries were dissected from the gravid and non-gravid horns, cannulated and pressurized to 10 mmHg. Concurrent changes in arterial diameter and $[Ca^{2+}]_i$ in response to the elevation of intraluminal pressure were studied using arteries loaded with the ratiometric Ca^{2+}-sensitive dye fura 2 AM (5 μM); Fura 2 fluorescence was assessed with a photomultiplier system. In a separate set of vessels, the endothelium was removed by passing air bubbles through the lumen and verified by the absence of dilation to acetylcholine.

Results: Elevation of pressure from 10 to 60 and 100 mmHg resulted in passive arterial distention of arteries from non-gravid horns without any significant change in $[Ca^{2+}]_i$. In contrast, arteries from gravid horns developed myogenic tone (17 ± 4 % at 60 mmHg; 20 ± 5 % at 100 mmHg) that was associated with an elevation in $[Ca^{2+}]_i$ from 80 ± 4 to 223 ± 45 and 336 ± 51 nM, respectively (n = 5). Synchronous oscillations in $[Ca^{2+}]_i$ and lumen diameter were frequently observed in vessels from gravid horns. Endothelial denudation did not alter myogenic behavior of uterine arteries from non-gravid horns.

Conclusion: Late pregnancy is associated with a striking enhancement of uterine artery reactivity to intraluminal pressure due to an up-regulation of cellular mechanisms that lead to elevations and oscillations in cytosolic $[Ca^{2+}]_i$. These adaptive changes in myogenic behavior are governed by local uteroplacental influences and are induced by the modulation of intrinsic properties of vascular smooth muscle cells independent of endothelial effects.

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196

Recurrent Miscarriage Is Associated with Abnormal Pre-Pregnancy Circulatory Function. Ineke Krabbendam, Marc EA Spaanderman, Frederik K Lotgering.* *Obstetrics/Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background:

Hemodynamic abnormalities prior to pregnancy predispose to subfertility, maternal gestational hypertensive sequelae and fetal growth restriction. In this study we tested the hypothesis that recurrent miscarriage (RM) is associated with abnormal pre-pregnancy circulatory function.

Method:
Sixty-one non-pregnant women with a history of RM (≥ 2 pregnancy losses prior to 16 weeks gestational age) were included in this study. Two women with chromosomal or uterine anomalies were excluded. All women were tested for overweight or obesity (body mass index 25-30 and >30 kg/m^2, respectively), thrombophilia (THROMB), hyperhomocysteinemia (HHC), abnormal plasma volume (PV; HSA I^{125} indicator dilution method, mean reference value ± 2 SD (ref) 48-64 ml/kg lean body mass), hypertension (oscilometrically determined blood pressure systolic >140 or diastolic >90 mmHg), heart rate (HR) and stroke volume (SV, Doppler echocardiography, ref 59-83 ml). From these data we calculated cardiac output (CO: SV*HR, ref 4.0-5.6 l/min), cardiac index (CI: CO/BSA, ref 2.2-3.4 l/min/m^2) and total peripheral vascular resistance (TPVR: 80*MAP/CO, ref 1000-1800 dyne.s/cm^5). Possible correlations were tested by Spearman's Rho. A p value < 0.05 was considered significant.
Results:
Twelve women (20%) had overweight and 7 women (12%) were obese. THROMB and HHC were present in 11 (19%) and 8 women (14%), respectively. Abnormal circulatory function was found in 46 women (78%). These included low PV (15/59, 25%), hypertension (5/59, 8%) and/or central hemodynamic abnormalities (37/59, 63%). Ten women (17%) had a hyperdynamic circulation with elevated CO and CI, and 18 women (31%) had a hypodynamic circulation with a low CO and CI, mostly in conjunction with a low SV (8/18; 44%). A high TPVR was observed in 10 women (17%). Further analysis showed that hemodynamic abnormalities were independent of THROMB or HHC.
Conclusion:
RM is often associated with a hyper- or hypodynamic circulation, with or without a low plasma volume, prior to pregnancy. We speculate that an abnormal pre-pregnancy circulatory function may interfere with implantation, placental development and/or uterine perfusion.

197

Angiotensin II, Angiotensin 1-7 and Their Relationship to Non-Pregnant Plasma Volume. Ira Bernstein,[*1] Adrienne Schonberg,[1] Robert Shapiro,[2] Beth Bouchard,[3] Alan Segal.[4] *[1]OB/GYN, University of Vermont, Burlington, VT, USA; [2]Neurology, University of Vermont, Burlington, VT, USA; [3]Biochemistry, University of Vermont, Burlington, VT, USA; [4]Medicine, University of Vermont, Burlington, VT, USA.*
BACKGROUND: Reduced plasma volume outside of pregnancy has been identified as a risk factor for the development of recurrent preeclampsia. We sought to determine the contribution of specific angiotensin hormonal end signals to plasma volume prior to a first pregnancy
METHODS: We have examined 39 healthy, reproductive age, nulligravid women during the follicular phase of the menstrual cycle to determine plasma volume and hormone profiles. All women were normotensive nonsmokers. Studies were performed after a minimum of 30 minutes of supine positioning during inpatient stays in our General Clinical Research Center. Prior to study subjects had 3 days of dietary control establishing sodium and calorie balance. During the hospital stay we measured plasma volume employing Evans blue dye dilution and plasma levels of angiotensin II and angiotensin $_{1\text{-}7}$. Data is expressed as mean $\pm$ standard deviation.
RESULTS: Subjects were 30.5 $\pm$ 4.6 years old with a BMI of 22.4 $\pm$ 3.6 kg/m^2 at the time of study. Mean plasma volume corrected for BMI was 129 $\pm$ 16 mL/kg/m^2. Mean AII and $A_{1\text{-}7}$ levels were 31.8 $\pm$ 11.0 and 4.6 $\pm$ 3.8 pg/mL respectively. The correlations between PV/BMI and AII, $A_{1\text{-}7}$ and the AII/$A_{1\text{-}7}$ ratio were AII: r = -0.16 (P = 0.35), $A_{1\text{-}7}$: r = -0.25 (P=0.15), AII/$A_{1\text{-}7}$ r= 0.06, (P =0.73).
CONCLUSIONS: Under normal resting non-gravid conditions, in this preliminary data, we have identified no significant association of Ang II, Ang $_{1\text{-}7}$ or the Ang II/ Ang $_{1\text{-}7}$ ratio to plasma volume corrected for body mass index. There is a tendency for both Ang II and Ang$_{1\text{-}7}$ to be inversely related to resting plasma volume. Supported by NIH RO-1 HL 71944.

198

Hyperandrogenemia Independent of Insulin Levels in the Third Trimester Gestational Diabetic with a History of Irregular Periods. Peter S Uzelac,[1] Steven T Nakajima,[1] Liqun Wu,[2] Frank Z Stanczyk.[*2] *[1]Obstetrics and Gynecology, University of Louisville, Louisville, KY, USA; [2]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA.*
OBJECTIVE: In the nonpregnant women a relationship between hyperinsulinism and hyperandogenemia is well established. Previously we have demonstrated increased insulin and total testosterone (T) levels in second trimester gestational diabetic (GDM) patients with a history of irregular menses. Because insulin resistance progresses with gestational age, our current study tested the hypothesis that GDM patients with a history of irregular menses continue to have increased insulin and androgens in the third trimester.
DESIGN: Two groups of pregnant women in the third trimester were studied. Group I (N=10) had a history of irregular periods (<6 periods/year) and a diagnosis of GDM based upon two or more elevated values on a 100-g 3-hour glucose tolerance test (GTT). Group II (N=11) had a history of regular periods (12 periods/year) and a diagnosis of GDM as just described.
MATERIALS AND METHODS: Fasting blood samples were obtained from all subjects. Serum levels of testosterone (T), dihydrotestosterone (DHT) and androstenedione (A) were quantified by RIA following organic solvent extraction and Celite column partition chromatography. Sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) and insulin were quantified by direct immunoassays. Free T values were calculated. Student's T test was used for statistical analysis.
RESULTS: BMI (35.83 vs. 34.42, p>.05), gestational age (31.7 wk vs. 31.8 wk, p>.05) and fasting insulin were similar between groups. A, free T and DHT were significantly elevated, whereas total T and DHEAS were nonsignificantly elevated and SHBG was nonsignificantly decreased in Group I.
CONCLUSION: Third trimester GDM women who have a history of irregular menses have higher androgen levels compared to those who have a history of regular periods. Since fasting insulin levels are similar between the groups, hyperandrogenemia in third trimester GDM women with a history of irregular periods may be related to factors other than hyperinsulinism.

Mean hormonal and SHBG levels

	Total T (ng/dl)	Free T (pg/ml)	A (ng/ml)	DHT (ng/ml)	DHEAS (mcg/ml)	Insulin (mcIU/ml)	SHBG (nmol/L)
GDM with irregular periods	120	6.58	3.54	.33	0.62	15.7	288
GDM with regular periods	81	3.37	1.62	.21	.43	15.4	369
p value	.12*	.03*	.01*	.04*	.12*	.96	.06

*One-sided test

199

Maternal Hepatic Fat Content Is Increased in Rats Undernourished during Lactation. Claudia J Bautista,[1] Hector Ledesma,[1] Peter W Nathanielsz,[*2] Elena Zambrano.[1] *[1]Dept. Reproductive Biology, Insituto Nacional de Ciencias Medicas y Nutricion SZ, Mexico DF, Mexico; [2]Center for Pregnancy and Newborn Research, Univ. TX Health Science Center San Antonio, Dept. Ob/Gyn, San Antonio, TX.*
OBJECTIVE. Several studies have been conducted on the effects of maternal nutrient restriction during pregnancy and/or lactation in the rat. These studies have been directed toward evaluation of offspring outcomes and less attention has been paid to potential direct effects on the mother. We have made the interesting observation that maternal hepatic liver fat content is increased in mothers eating a restricted low protein diet during lactation. Our aim was to evaluate whether this extra maternal hepatic adipose deposition could be related to maternal leptin concentration. **METHODS.** Pregnant rats were assigned to control (C) (20% casein; CC) or a restricted (R) (10% casein; RR) isocaloric diet in pregnancy (first letter) and lactation (second letter). A third group received C and R in pregnancy and lactation, respectively (CR). A fourth group received R in pregnancy and C in lactation (RC). Maternal serum leptin at PND 25 was measured by RIA. Maternal hepatic fat was determined by Soxtec method. Data are mean ± SEM, analysis was by the Pearson correlation. **RESULTS.** Both groups restricted during lactation, RR and CR had higher maternal hepatic fat in comparison with CC and RC (p<0.05) Fig 1A. RC group had lower serum leptin (p<0.05) than CR (Fig 1B). **CONCLUSIONS.** Hepatic fat deposition was increased in both groups undernourished during lactation. There are very few data on production of leptin by hepatic cells or in relation to hepatic in distinction to adipose tissue fat. Further studies are needed to determine the relative contributions of adipose tissue and liver to circulating maternal leptin.

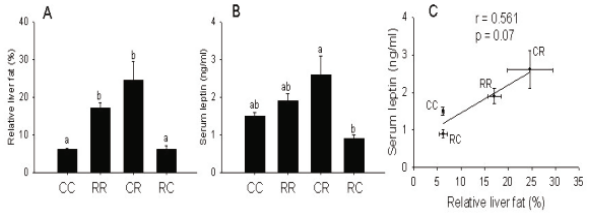


Fig 1. Maternal (A) relative liver fat (%), (B) serum leptin and (C) serum leptin as a function of relative liver fat on PND 25 in the four groups exposed to different diets during pregnancy and lactation (CC: control-control; RR: restricted-restricted; CR: control-restricted; RC: restricted-control). Mean±SEM; n=5, data not sharing a letter are different, p<0.05.

200

Coding Variants in *PXR* in Intrahepatic Cholestasis of Pregnancy; Effect on Target Gene Activity. Peter H Dixon,[1] Bryn M Owen,[1] Saskia WC van Mil,[1] Roger White,[1] Elwyn Elias,[2] Catherine Williamson.[1] (SPON: Phillip R Bennett). *[1]Maternal and Fetal Disease Group, IRDB, Imperial College, London, United Kingdom; [2]Liver Unit, University of Birmingham Trust Hospital, Birmingham, West Midlands, United Kingdom.*

Intrahepatic cholestasis of pregnancy (ICP) has a complex aetiology, with genetic and hormonal components. The pregnane-X-receptor (PXR) is a ligand-activated transcription factor responsible for regulating target gene expression involved in detoxification of xenobiotic and endobiotic metabolites and is a key player in bile acid metabolism. 13 non-synonymous single nucleotide polymorphisms (SNPs) have been reported in PXR, some of these alter the ability of the protein to bind to and activate PXR target genes

Objectives: We hypothesised that:

1) Coding region mutations of *PXR*, and/or a target gene involved in bile acid metabolism, *SULT2A1* are associated with ICP.

2) *PXR* variants influence the response to therapeutic ligands and to the hydrophobic bile acid LCA.

Methods: 1) The coding regions of *PXR* and *SULT2A1* were amplified and sequenced in 125 patients using an Applied Biosystems 3100 genetic analyser.

2) HepG2 cells were seeded into 96 well plates and transfected with expression vectors for wild-type or mutant *PXR*, a *PXR* responsive *CYP3A4* Luciferase reporter gene and an internal control vector, pRL-CMV. Cells were treated for 24h with PXR ligands and assayed for Luciferase activity.

Results: 1) We identified 2 non-synonymous SNPs in *PXR*, G36R and P27S (in 8 and 1 cases respectively) and a single non-synonymous SNP (A261T) in *SULT2A1*. These variants occur in accordance with the published allele frequencies.

2) Of the 13 known SNPs in the *PXR gene*, four (R98C, V140M, Q158K, D163G) were observed to have altered transcriptional activation of the *CYP3A4* promoter in the presence of Rifampicin, Ursodeoxycholic acid, Dexamethasone and LCA. However, no differential response was observed for the G36R and P27S SNPs that are present in UK ICP cases.

Conclusions: 1) Coding region mutations of *PXR* and *SULT2A1* are not associated with ICP in UK cases.

2) Our analysis is the first comprehensive study of functional PXR variants in a single model system. We demonstrated altered activation of *CYP3A4* in the presence of 4 PXR ligands (drugs used to treat ICP and LCA). However, *CYP3A4* activation did not change with the PXR variants that were demonstrated in UK ICP cases, indicating that these variants are not relevant to treatment response in our population.

201

Heterozygous *ABCB11 (BSEP)* Mutations in Patients with Intrahepatic Cholestasis of Pregnancy. Peter H Dixon,[1] Saskia WC van Mil,[1] Jennifer A Chambers,[1] Sandra S Strautnieks,[2] Richard J Thompson,[2] Frank Lammert,[3] Anna G Glantz,[4] Lars-Ake Mattsson,[4] Catherine Williamson.[1] (SPON: Phillip R Bennett). *[1]Maternal and Fetal Disease Group, IRDB, Imperial College, London, United Kingdom; [2]Department of Liver Studies and Transplantation, Kings College School of Medicine, London, United Kingdom; [3]Internal Medicine I, University Hospital Bonn, Bonn, Germany; [4]Department of Obstetrics and Gynaecology, Sahlgrenska Hospital, East Goteborg, Sweden.*

Intrahepatic Cholestasis of Pregnancy (ICP) has a complex aetiology with a significant genetic component. Two genes that are mutated in progressive familial intrahepatic cholestasis (PFIC) (*ABCB4, ATP8B1*) have been implicated in the pathogenesis of the disease. Heterozygous mutations of these genes have proved to be a rare cause of predisposition to ICP. The third PFIC gene, *ABCB11*, encodes the bile salt export pump (BSEP) and homozygous mutations cause a spectrum of disease ranging from PFIC to benign recurrent intrahepatic cholestasis (BRIC).

Objectives. We sought to clarify the role of *ABCB11* mutations in ICP by screening 369 OC UK cases for two common European mutant alleles (E297G, D482G) by PCR amplification and DNA sequencing. In addition a second cohort of 158 ICP patients from Sweden and Germany were subject to the same analysis.

Methods. PCR primers from the UCSF website (http://pharmacogenetics.ucsf.edu/set1/index.html) were used to amplify and sequence patient DNA using an Applied Biosystems 3100 genetic analyser. Electropherograms were analysed by the use of Codoncode Aligner software, and results checked by an independent observer.

Results. Inspection of the two exons of the *ABCB11* gene harbouring the known common mutations in the first cohort identified three occurrences of the E297G mutation. In the second cohort, a single occurrence of each mutation (E297G and D482G) was identified.

Conclusion. Heterozygosity for the common *ABCB11* mutations are likely to represent a rare predisposition to ICP in the UK and European cohorts, accounting for only 1% of cases.

202

Differential Nogo-B Expression in the Human Cycling Endometrium and the Decidua. Graciela Krikun,[*] Charles J Lockwood,[*] Seth M Guller.[*] *Ob/Gyn & Rep. Sciences, Yale University, School of Medicine, New Haven, CT, USA.*

Introduction: Several angiogenic factors are believed to be involved in physiologic as well as pathologic angiogenesis in the human endometrium. Recent findings have demonstrated that neurite outgrowth factor-B (Nogo-B), a member of the reticulon 4 family normally involved in axonogenesis, appears to play a critical role as regulator of cell migration and vascular remodeling. The N-terminus of Nogo-B promotes migration of endothelial cells but inhibits migration of vascular smooth muscle cells. Vascular injury in Nogo-A/B-deficient mice promoted exaggerated neointimal proliferation, (Nat Med. 2004). This led us to investigate whether Nogo-B is present in human pregnant and non-pregnant endometrium, a tissue requiring continuous angiogenesis in the adult.

Methods: After obtaining informed consent, endometria from reproductive age women were obtained from hysterectomies for benign conditions. Portions of endometrium or decidua were formalin-fixed and paraffin-embedded for immunohistochemical studies. The remainder were used for cell culture, protein and mRNA analysis.

Results: Initial immunohistochemical studies now demonstrate that Nogo-B is widely but differentially expressed throughout the pregnant and non-pregnant endometrium. Of special interest, it was observed that Nogo-B expression was essentially absent from the glandular epithelium during the proliferative phase of the cycle but highly expressed in the secretory phase and throughout pregnancy. We confirmed these findings in vitro both at the mRNA and protein levels using cultured endometrial stromal, endothelial, or glandular epithelial cells. Western blotting revealed that Nogo-B was detected at the predicted MW of 50 kD in stromal cells and endothelial cells in the presence or absence of steroid hormones. Consistent with results of the immunohistochemical studies, Western blot analysis and quantitative RT-PCR demonstrated that as in the proliferative phase, glandular epithelial cells cultured without progestins did not express Nogo-B.

Conclusion: While several angiogenic factors have been identified and are believed to be involved in physiological as well as pathological angiogenesis in the human endometrium, we propose that Nogo-B may be a critical angiogenic factor in endometrial and uteroplacental vascular development. The regulation of Nogo-B in the endometrial glandular epithelium suggests that the protein may be essential in the process of decidualization.

203

Relationship between Bone Mineral Density and Parity in a Multiethnic Sample of Healthy Reproductive-Aged Women. Jennifer L Newman, Carmen Radecki Breitkopf, Abbey B Berenson.[*] *Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: To examine the relationship between bone mineral density (BMD) and parity among healthy, multiethnic reproductive-aged women.

Methods: A total of 707 African American, Hispanic, and Caucasian women aged 16-33 years who participated in a longitudinal study on BMD and contraceptive use completed questionnaire items assessing demographic and behavioral correlates of bone density. Bivariate associations were analyzed using Pearson and Spearman r, χ^2 test, and ANOVA. In addition, multivariate regression analyses were performed to evaluate the effect of parity on BMD at the lumbar spine (L1-L4) and dominant hip while adjusting for race/ethnicity, age, age at menarche, body mass index (BMI), and tobacco use.

Results: At the bivariate level, race/ethnicity and parity (0, 1, and ≥ 2) were independently associated with lumbar spine BMD ($p < .05$). The relationship between parity and dominant hip BMD was non-significant. Further analyses demonstrated that the relationship between parity and lumbar spine BMD was moderated by race/ethnicity ($p < .05$). In the multivariate analysis, the main effect of parity on lumbar spine BMD diminished after adjusting for key clinical covariates.

Conclusions: Our findings suggest that while race/ethnicity remains an important factor, parity does not have an appreciable effect on BMD levels among reproductive-aged women.

203.1

Vascular Endothelial Growth Factor (VEGF) Is a New Player in the Slow Relaxin (Rlx) Vasodilatory Pathway. Julianna E Matthews,[1] J Peter Rubin,[2] Jacqueline Novak,[3] Kirk P Conrad.[*4] *[1]Magee Womens Research Institute, Pittsburgh, PA, USA; [2]Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; [3]Department of Biology, Walsh University, Canton, OH, USA; [4]Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*

The vasodilatory mechanisms of Rlx vary according to the duration of exposure to the hormone. After day(s) of recombinant human (rh) Rlx administration to rats, vascular (matrix metalloproteinase) MMP-2 activity is increased which processes big (endothelin) ET to ET_{1-32} at a gly-leu bond, that in turn, activates the endothelial ET_B receptor/NO vasodilatory pathway (KP Conrad, J Novak Am J Physiol 287:R250, 2004). After hour(s) of exposure, vascular MMP-9 rather than MMP-2 activity is increased and serves as the endothelin converting enzyme (A Jeyabalan et al. Endocrinol, in press). We hypothesized that these slow vasodilatory responses to Rlx require bi-directional communication between the endothelium and vascular smooth muscle involving VEGF (VSM → EC) and NO (EC → VSM). Small rat renal and human subcutaneous arteries were mounted in a pressure arteriograph and myogenic reactivity was investigated (% change in diameter over baseline in response to a 20 mmHg step increase in intraluminal pressure). After incubation with 30 ng/ml rhRlx for 3 h *in vitro*, there was a significant reduction in myogenic reactivity that was prevented following incubation with 0.1 mM L-NMA, 10 mM RES-701-1 (an ET_B receptor antagonist) or 1 mM GM6001 (a general MMP inhibitor). Pre-treatment with the VEGF receptor tyrosine kinase inhibitor, SU5416 (3mM) prevented the decrease in myogenic reactivity induced by rhRLX ($p<0.004$ vs dilute DMSO vehicle). A comparable inhibitory response was observed after instillation of 3 mg/ml VEGF neutralizing antibody in the artery lumen ($p<0.002$ vs goat IgG). In contrast, post-treatment with SU5416 did not block the vasodilatory action of Rlx. In preliminary studies, pre-treatment with either SU5416 or VEGF neutralizing antibody also blocked the reduction in myogenic activity by rhRlx in the human subcutaneous arteries. Conclusions: (i) Incubation of small arteries with rhRlx *in vitro* inhibits myogenic reactivity via a vascular MMP/ET_B/NO pathway; (ii) VEGF is a new player in the vasodilatory pathway that is most likely in series with, and upstream of, vascular MMP-9 or -2.

203.2

Relaxin (Rlx) Induces Fast Relaxation in Some Rat and Human Arteries Mediated by PI3 Kinase and Nitric Oxide. Julianna E Matthews,[1] J Peter Rubin,[2] Jacqueline Novak,[3] Kirk P Conrad.[*4] *[1]Magee Womens Research Institute, Pittsburgh, PA, USA; [2]Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; [3]Department of Biology, Walsh University, Canton, OH, USA; [4]Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesvillle, FL, USA.*

The vasodilatory mechanisms of Rlx vary according to the duration of exposure to the hormone. Fast relaxation responses to Rlx were reported for human pre-constricted gluteal, but not pulmonary arteries, that were abolished by endothelial denudation (C. Fisher et al. Circ. 106:292-5, 2002). We studied small coronary, mesentery and renal arteries from rats, as well as human subcutaneous arteries isolated from fat obtained after abdominal reductions. The arteries were mounted in a pressure arteriograph, and after pre-constriction to EC_{50} with phenylephrine, they were incubated with increasing concentrations of recombinant human relaxin (rhRlx) from 1 to 100 ng/ml (maximum relaxation response < 5 min). Of the 3 types of rat arteries investigated, only the small renal arteries demonstrated a concentration dependent relaxation ($p<0.001$ by ANOVA) with a maximum response of 20-30% relaxation at the highest dose of rhRlx. This relaxation response was abolished by prior endothelial removal or by pre-incubation with 0.1mM L-NMA ($p<0.001$ by ANOVA with vs without L-NMA). Pre-treatment with the PI3 kinase inhibitors, LY294002 (3mM) or Wortmannin (10nM), attenuated the fast relaxation response by ~ 80% ($p<0.05$ by ANOVA vs dilute DMSO vehicle). Human subcutaneous arteries also demonstrated a concentration dependent relaxation ($p<0.001$ by ANOVA) with a maximum response of 60-80% relaxation at the highest dose of rhRlx. In preliminary studies, the rapid vasodilatory response to rhRlx in human subcutaneous arteries was completely inhibited by both L-NMA and LY294002. In conclusion, some rat and human arteries show a fast relaxation response to relaxin mediated by PI3 kinase and NO.

204

A Comparison of Visceral and Subcutaneous Adipocyte Function in Healthy Pregnant Women. Shahzya S Huda,[1] Eng K Tan,[1] Colin Perry,[2] Ian Greer,[*1] Anna F Dominiczak,[3] Dilys J Freeman,[1] Naveed Sattar.[1] *[1]Division of Developmental Medicine, Glasgow University, Glasgow, United Kingdom; [2]Department of Endocrinology, Glasgow Royal Infirmary, Glasgow, United Kingdom; [3]BHF Cardiovascular Research, BHF Cardiovascular Research Centre, Glasgow, United Kingdom.*

Introduction: Pre-eclampsia (PE) is a major cause of maternal morbidity and mortality. Obesity is a key risk factor for this disease. Women with PE have an early exaggerated rise in triglycerides and free fatty acids (FFA). The cause of these lipid abnormalities is not known but disordered adipocyte function in women with PE may be a contributor to the pathogenesis of this syndrome.

Methods: Subcutaneous adipose samples were obtained from 11 pregnant women with paired visceral samples in 10. Adipocyte suspensions were prepared and incubated with isoproterenol ($2x10^{-7}$ M), insulin (10nM) and isoproterenol with insulin for two hours. Lipolysis rates were determined by measuring release of FFA and glycerol in an aliquot of incubation medium. Cell diameter was determined by direct microscopy of fresh cells in suspension.

Results: Subcutaneous adipocytes (SA) were significantly larger than visceral adipocytes (VA) [104.5um (13.8) vs 78.9 um (17.9) $p=0.002$]. BMI correlated with both visceral fat cell size ($r=0.81$, $p=0.004$) and subcutaneous fat cell size ($r=0.63$, $p=0.036$). In VA isoproterenol has a stimulatory effect (mean increase 216% $p=0.013$) and insulin an inhibitory effect (mean decrease 51% $p=0.0040$) on basal lipolysis rates. Insulin attenuated the effect of isoproterenol stimulated lipolysis (mean decrease 67.4% $p=0.002$). In SA there was no significant inhibition of basal release of FFA ($p=0.09$) and glycerol ($p=0.47$) by insulin. The degree of isoproterenol stimulation of lipolysis is inversely correlated to the basal lipolysis rate in SA($r=-0.82$ $p=0.002$) and VA ($r=-0.799$ $p=0.006$). When corrected for fat cell size there was no significant difference in basal, isoproterenol stimulated or insulin inhibited lipolysis rates between VA and SA.

Conclusion: Metabolic properties of adipocytes are related to both regionality of the tissue and cell size, with visceral adiposity and increasing adipocyte size predisposing to adverse metabolic profiles. Increasing BMI in healthy pregnant women is associated with increasing adipocyte size in both visceral and subcutaneous adipose depots. This may be an important mechanism through which obesity leads to altered adipocyte function and may predispose to pre-eclampsia.

205

The Effect of Clinical Intervention with the PDE5 Inhibitor Sildenafil Citrate (Viagra®) on Small Arteries from Women with Pre-Eclampsia. Rebekah A Samangaya, Mark Wareing, Philip N Baker.[*] *Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Hypothesis Pre-eclampsia is a multi-system disorder with an underlying pathophysiology of endothelial dysfunction. In vitro, myometrial small arteries show reduced endothelial-dependent relaxation, which is improved in the presence of phosphodiesterase type 5 (PDE5) inhibitors. Clinical intervention with the PDE5 inhibitor sildenafil citrate (Viagra®) would therefore be expected to improve relaxation of small myometrial arteries and thus improve uteroplacental blood flow.

Methods Myometrial and omental biopsies (N) were taken from women participating in a multi-centre double-blinded randomised placebo controlled trial of sildenafil in women with pre-eclampsia before 34 weeks gestation. Small arteries (n) were dissected free and mounted on wire myographs gassed with 20, 5 and 2% oxygen. Contraction was assessed with vasopressin (AVP; max. 10^{-8}M) and endothelial-dependent relaxation with bradykinin (BK; 10^{-10}-10^{-6}M).

Results 7 myometrial biopsies from each group, 8 omental biopsies from women taking sildenafil and 6 omental biopsies from women taking placebo were collected. Myometrial contraction was not altered by clinical intervention with sildenafil (Table 1; ANOVA). Similar data was seen with omental arteries. At 20% oxygenation, maximal BK-induced myometrial arterial relaxation was not significantly different with sildenafil vs. placebo (46±10% vs.40±12 %; $P>0.05$ ANOVA). Similarly, no difference was seen at 5 or 2% oxygenation. Omental arterial relaxation was significantly improved 2% vs 20% oxygen in sildenafil and placebo groups ($P<0.05$; 2-way ANOVA). Relaxation of omental arteries from women taking sildenafil was not significantly improved vs. placebo ($P>0.05$; 2-way ANOVA).

Conclusions Clinical intervention with sildenafil did not improve the endothelial-dependent relaxation in myometrial or omental small arteries. Pharmacokinetic data suggested reduced plasma sildenafil compared to healthy volunteers. Additionally, the final sildenafil dosage was often many hours pre-myography. Thus, acute effects of sildenafil may not have been present at the time of investigation.

Maximal constriction (kPa) evoked by AVP in myometrial vessels

Oxygenation	Sildenafil: Mean±SEM; n (N)	Placebo: Mean±SEM; n (N)
20% O_2	10.9±2.9; 13 (7)	11.6±2.8; 13 (7)
5% O_2	12.0±2.5; 14 (7)	11.2±3.1; 14 (7)
2% O_2	10.0±2.7; 13 (7)	13.9±1.2; 13 (7)

206

Arterial Pulse Wave Analysis in Women with Pre-Eclampsia, Pregnancy Induced Hypertension and Essential Hypertension with Superimposed Pre-Eclampsia. Asma A Khalil, Derek J Cooper, Kevin F Harrington. (SPON: Donald M Peebles). *Obstetrics and Gynaecology, Homerton University Hospital, London, United Kingdom.*

Objective: To compare arterial pulse wave analysis in women with pre-eclampsia (PE), those with pregnancy induced hypertension (PIH) and those with essential hypertension with superimposed PE (HT).

Background: Vascular compliance can be measured by analyzing the arterial pulse waveform obtained using applanation tonometry. Although commonly used outside pregnancy to investigate central hemodynamics in cardiovascular disease, few studies have used this technique in pregnancy. Our previous work in this field has confirmed that vascular compliance is reduced in PE compared with normal pregnancy.

Methods: Using applanation tonometry, radial artery pulse waveform was recorded in 21 women with PE, 21 with PIH and 23 with HT. From this, an averaged aortic waveform was calculated. Augmentation pressure (AP) and Augmentation Index at heart rate 75/min (AI-75) – measures of vascular compliance - were calculated.

Results: Oneway ANOVA testing showed highly significant differences between the groups for AP and AI-75. Pairwise comparisons showed highly significant differences between the PE and PIH groups [Mean differences (95% CI) were AP: 6.7 (2.6, 10.8); AI-75: 12.8 (6.5, 19)], and between the PE and HT groups [Mean differences (95% CI) were AP: 6.6 (2.6, 10.6); AI-75: 13.5 (7.4, 19.6)].

Conclusions: This study using a novel technique confirms that vascular compliance is reduced in women with PE compared with PIH or HT. This technique has promise for the clinical assessment and treatment of hypertensive disorders of pregnancy, and to enable research into the hemodynamic changes associated with these disorders.

207

Arterial Pulse Wave Analysis in Women with Pre-Eclampsia before and after Antihypertensive Treatment Compared with Non-Pregnant Women. Asma A Khalil, Derek J Cooper, Kevin F Harrington. (SPON: John J Morrison). *Obstetrics and Gynaecology, Homerton University Hospital, London, United Kingdom.*

Objectives

1. To compare arterial pulse wave analysis in women with pre-eclampsia with both normal pregnant and non-pregnant women. 2. To ascertain the effects of antihypertensive treatment on arterial pulse wave analysis in women with pre-eclampsia.

Background

Vascular compliance can be measured using applanation tonometry. One report suggests that this technique can distinguish the vascular changes of pre-eclampsia (PE) from those of normal pregnancy. Our previous work using applanation tonometry has shown that vascular compliance is increased in normotensive pregnant compared with non-pregnant women.

Methods

Using applanation tonometry, radial artery pulse waveform was recorded in 35 women with PE (repeated after antihypertensive treatment when applicable), a similar number of gestational age-matched controls, and 20 healthy non-pregnant women. From this, an averaged aortic waveform was calculated. Augmentation pressure (AP) and Augmentation Index at heart rate 75/min (AI-75) – measures of vascular compliance - were calculated.

Results

Mean AP (11.9 versus 3.0) and mean AI-75 (32.3 versus 16.8) were significantly higher in pre-eclamptic compared with normotensive controls ($p<0.001$), and pre-eclamptic compared with non-pregnant women (11.9 versus 6.8, $p=0.009$; and 32.3 versus 24.6, $p=0.011$). Antihypertensive treatment did not significantly affect mean AP (12.9, 11.9) or mean AI-75 (33.3, 30.1).

Conclusion

This study confirms that vascular compliance is reduced in pre-eclamptic women compared with normotensive controls and non-pregnant women; vascular compliance was not improved by antihypertensive treatment. This technique has promise for the assessment and treatment of PE. The potential for early prediction of PE should also be explored.

208

Preeclampsia and Neonatal Outcomes in Near-Term Infants. Angie Child,[1] Anjali Kaimal,[1] Norton Mary,[1] Michael Kuzniewicz,[2] Caugey B Aaron.[1] (SPON: Linda C Giudice). *[1]Obstetrics and Gynecology, UCSF, San Francisco, CA, USA; [2]Pediatrics, UCSF, San Francisco, CA, USA.*

Objective: To evaluate neonatal outcomes in near-term infants delivered due to pre-eclampsia

Design: A retrospective cohort of 3,580 infants delivered at 32 0/7 to 36 6/7 weeks gestation was examined. Neonatal outcomes of 499 infants who delivered prematurely due to preeclampsia were compared with outcomes of 3,081 infants delivered prematurely due to other etiologies. Outcomes included: small for gestational age (SGA), neonatal intensive care unit (NICU) admission, and neonatal death. Multivariate logistic regression was used to analyze the association between preeclampsia and the neonatal outcomes, controlling for potential confounders.

Results: Infants of women with preeclampsia were more likely to be SGA and admitted to the NICU; however, they were less likely to suffer a neonatal death.

Neonatal outcomes

	SGA	NICU Admission	Neonatal Death
Preeclampsia	51.3	54.3	2.2
Other Term Deliveries (%)	4.3	39.0	3.4
Adjusted OR (95% CI)	3.7 (2.8-4.8)	1.3 (1.0-1.7)	0.4 (0.2-0.8)

Conclusion:

Neonatal outcomes in near-term infants born to preeclamptic mothers are significantly different from outcomes in near-term neonates delivered due to other indications. Infants born to preeclamptic mothers were more likely to be small for gestational age, but had a decrease in mortality. This may be a reflection of the differences in the underlying pathophysiology behind indicated preterm birth due to preeclampsia and preterm birth due to other etiologies.

209

Autophagy-Related Proteins in Placentas from Pregnancies Complicated with Preeclampsia and Intrauterine Fetal Growth Restriction. Soo-young Oh,[1] Suk-Joo Choi,[1] Eun Yoon Cho,[2] Cheong-Rae Roh.[1] (SPON: Yoel Sadovsky). *[1]Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.*

Objective: To investigate the expression of autophagy-related proteins (beclin-1 and LC3) in human placenta and their changes in placentas from pregnancies complicated with preeclampsia or intrauterine fetal growth restriction

Material and methods: Human term placentas were collected after delivery from women with a normal pregnancy (n=6), preeclampsia (PE, n=10), and intrauterine fetal growth restriction (IUGR, n=8). Direct visualization of autophagosome was performed with electron microscopy. The expression of beclin-1 and LC3 was assessed by immunofluorescence, immunohistochemistry and Western blotting.

Results : Using electron microscope, we confirmed the presence of autophagosome in trophoblasts of the human placenta. Beclin-1 and LC3 were well expressed in human placenta. The expression of beclin-1 in placenta was decreased in IUGR and PE, compared to normal pregnancy. Importantly, the

expression of LC3 was decreased in placentas from IUGR and increased in PE compared with normal controls. Moreover, massive proteinuric PE (> 10 g/d) was associated with a striking increase in the expression of placenta LC3.

Conclusions : The human placenta expresses autophagosome and autophagy-related proteins such as beclin-1 and LC3. The difference in the expression of autophagy-related proteins between PE and IUGR may be related to the pathophysiology of these conditions.

Figure 1. Representative western blot of beclin-1 and LC3 in placentas of normal pregnancy, preeclampsia, and intrauterine growth restriction

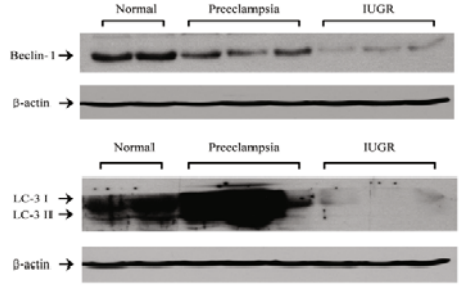
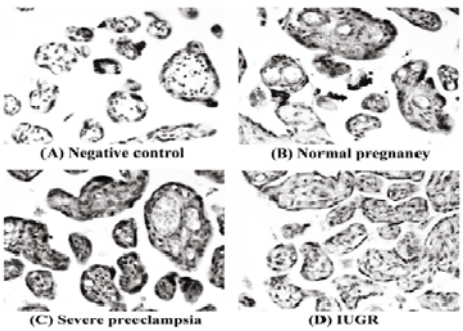


Figure 2. LC3 expression in human placenta by IHC.



210

Interleukin-6, Tumor Necrosis Factor-α, Lipid Peroxide Levels, Total Antioxidant Ability, and Antioxidant Vitamin Levels in the Umbilical Venous Plasma of Preeclampsia. Yoon Ha Kim,[*1] Tae-Bok Song,[1] Cheol H Kim,[1] Sung Y Yang,[2] Bong W Ahn.[2] *[1]Ob/Gyn, Chonnam National University Medical School, Gwangju, Republic of Korea; [2]Biochemistry, Chonnam National University Medical School, Gwangju, Republic of Korea.*

Objective: Our purpose was to investigate Interleukin-6 (IL-6), tumor necrotic factor-α (TNF-α), lipid peroxides levels, oxygen-radical absorbance capacity (ORAC), and antioxidant vitamin levels in umbilical venous plasma and to evaluate the roles of them in the pathophysiology of preeclampsia.

Study design: Samples of umbilical venous plasma were obtained from 20 normal and 20 preeclamptic women between 33 and 40 weeks gestation. IL-6 and TNF-α were assayed by an enzyme-linked immunoassay. Lipid peroxide levels were measured by thiobarbituric acid reaction. The ORAC values were measured by Cao's method. Ascorbic acid, retinol, α-tocopherol, and γ-tocopherol were measured by high performance liquid chromatography.

Results: There was no significant differences of IL-6 levels in umbilical venous plasma between women with normal and preeclampsia (2.79±0.21 vs. 2.94±0.17 pg/ml). TNF-α levels in umbilical venous plasma of women with preeclampsia were significantly higher than that of women with preeclampsia (3.04±0.01 vs. 1.40±0.01 pg/ml, $p<0.01$). Lipid peroxide levels in umbilical venous plasma of women with preeclampsia were significantly higher than that of women with normal pregnancy (7.32±0.09 vs. 5.18±0.14 nmol/mg protein, $p<0.01$). The ORAC values in umbilical venous plasma of women with preeclampsia were significantly lower than that of women with normal pregnancy (12836.5±249.4 vs. 10490.2±276.9 U/ml, $p<0.05$). Ascorbic acid levels in umbilical venous plasma of women with preeclampsia were significantly lower than those of women with normal pregnancy (320.2±48.5 vs. 538.5±68.2 nmol/ml, $p<0.05$).

Conclusion: These findings suggest that increased TNF-α in placenta may induce oxidative stress origin and increased lipid peroxidation and decreased antioxidant activity in placenta is involved in the pathophysiology of preeclampsia. An antioxidant vitamin, ascorbic acid, may act an important antioxidant factor and be involved antioxidant ability of fetus in preeclampsia.

211

Measures of Vascular Dysfunction Persist Months Postpartum after Preeeclampsia. Robert W Powers,[*1,2] Marcia J Gallaher,[1] Ashlie Prioleau,[1] Gail M Harger,[3] Nina Markovic,[1,3] James M Roberts.[*1,2,3] *[1]Magee-Womens Research Institute; [2]Obstetrics & Gynecology; [3]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA.*

Objective: Preeclampsia and cardiovascular disease share many risk factors and women with preeclampsia are at increased risk of cardiovascular mortality later in life. We investigated whether two measures associated with vascular dysfunction that are elevated in preeclampsia, cellular fibronectin (cFN) and uric acid, would remain higher months postpartum.

Methods: We measured plasma cFN and uric acid in 30 women with uncomplicated normotensive pregnancies and 20 women with preeclampsia in samples collected at both predelivery and several months postpartum. cFN was quantified by ELISA, inter-assay CV=7%. Uric acid was determined by commercial kit, inter-assay CV=4.5%. Data are mean±SD. Statistical analysis was by one-way and paired Students t-tests with statistical significance accepted at $p<0.05$.

Results: The mean concentration of cFN and uric acid were both significantly elevated in plasma samples collected predelivery from women with preeclampsia compared to women with uncomplicated pregnancies ($p<0.0001$ for both). cFN was still higher months postpartum in women with previous preeclampsia compared to women with a previous uncomplicated pregnancy ($p<0.05$). Similarly, uric acid was also still higher months postpartum in women with previous preeclampsia compared to women with a previous uncomplicated pregnancy ($p=0.01$). In paired analysis, both cFN ($p<0.01$) and uric acid ($p<0.01$) exhibited a significant decrease in concentration postpartum in preeclampsia cases; however, only cFN exhibited a significant decrease postpartum ($p<0.05$) in uncomplicated controls.

Conclusions: Uric acid and cFN are both higher in preeclampsia compared to uncomplicated pregnancy and are still higher months postpartum. These data suggest subtle vascular dysfunction and metabolic differences in women with previous preeclampsia consistent with the increased risk of future cardiovascular disease in these women.

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	Uncomplicated pregnancy controls (n=30)		Preeclampsia cases (n=20)	
	Pre-delivery	Postpartum	Pre-delivery	Postpartum
Gestational age at delivery (weeks)	39.7±1.4		34.9±3.9*	
Months postpartum		8.9±4.2		13.2±7.8*
cFN (μg/ml)	41.1±21.6	30.3±17.1	86.4±44.0*	43.8±26.1*
Uric acid (mg/dl)	4.8±0.9	4.5±1.2	6.7±1.4*	5.3±0.8*

212

Sildenafil Does Not Prolong Pregnancy in Women with Established Pre-Eclampsia: Results of a Phase 2 Randomised Placebo Controlled Trial. Rebekah Smith,[2] Laurence Skillern,[1] Gary Mires,[3] Andrew Shennan,[4] Alison McLeod,[1] Ian Hirsch,[1] David Howe,[1] Philip Baker.[*2] *[1]Pfizer Global Research & Development, Sandwich, United Kingdom; [2]University of Manchester, St Mary's Hospital, United Kingdom; [3]University of Dundee, Ninewells Hospital, United Kingdom; [4]King's College London, St Thomas's Hospital, United Kingdom.*

Background: Deficient placentation and reduced spiral arteriole blood flow is believed to be important in the pathogenesis of pre-eclampsia (PET). Pre-clinical evidence suggests that the phosphodiesterase 5 inhibitor sildenafil can increase uterine artery blood flow. We report a clinical study of the effect of sildenafil in women with established PET testing the null hypothesis that sildenafil does not prolong pregnancy.

Methods: Women with a singleton pregnancy between 24-34 weeks gestation who developed new onset proteinuria of ≥ 500 mg/24 h with hypertension were randomised to placebo or sildenafil (20 mg tid, escalating to 40 mg tid after 3 days and to 80 mg tid after a further 3 days). Women for whom urgent delivery (within 24 h) was planned were excluded. Concomitant treatment with antihypertensives was allowed. Delivery was permitted for a pre-specified set of clinical criteria. The primary endpoint was time from randomization to delivery. The log transformed primary endpoint was analysed by ANOVA with gestational age at randomization (<28 w or ≥28 w), presence or absence of IUGR and treatment as factors. Calculations suggested a sample size of 58 subjects was required to detect a difference of ≥ 5 days between groups. The trial was stopped after the first interim analysis when the SD of the primary endpoint was found to be less than expected and simulations indicated the study already had >95% power.

Results: 35 subjects contributed to the modified intention to treat sample set. Overall, the ratio of the Log transformed primary endpoint for sildenafil/placebo was 0.86 with 95% CI of 0.47-1.58. The confidence interval includes unity indicating no difference between groups. Gestation or the presence of IUGR did not affect the result. Table summarises days to delivery by treatment group and gestational age.
Conclusion: We conclude that sildenafil in the dose range of 20 mg TID to 80 mg TID is ineffective for the prolongation of pregnancy in established PET.

Treatment	Sildenafil		Placebo	
Gest age	<28 w	>28 w	<28 w	>28 w
Number	5	12	6	12
Days to delivery (mean (95%CI))	5.2 (1.2-9.3)	5.7 (2.8-8.6)	6.2 (1.6-10.9)	7.5 (1.9-13)

213

Absent and Reverse End Diastolic Umbilical Doppler and Placental Annexin V Expression: Consequences on the Neonatal Hematologic Status. Gabriele Urban,[1] Patrizia Vergani,[1] Frncesca Sala,[1] Cristina Farina,[2] Valentina Dell'Orto,[2] Biagio Eugenio Leone.[3] (SPON: Charles J Lockwood). *[1]Obstetrics and Gynecology, University of Milan Bicocca, Monza, Italy; [2]Pediatrics, University of Milan Bicocca, Monza, Italy; [3]Pathology, University of Milan Bicocca, Monza, Italy.*
Background: Annexin V disruption on the placental endotelil surface is thought to be associated with pathogenesis in intrauterine growth restriction (IUGR), thrombin formation on damaged endothelium in antiphospholipid syndrome (APS) in pre-eclampsia and the haemolysis, elevated liver enzyme and low platelet count (HELLP) syndrome leads to the activation of platelets in maternal circulation. We evaluates the relationship between fetal Doppler evaluation, annexin V expression and neonatal hematologic parameters.
Study design: Prospective cohort Placental sample were performed at three different point of placental bed, marginal, central and close to the insertion of the umbilical cord, samples were stored in double at -80º C and in formaline. Maternal and fetal blood sample were collected respectively from antecubital vein and umbilical artery. Immuno Hystochemical analysis was performed blinded to the clinical condition. Samples were immunostained in 10 slides, in each slide IHC reactivity was counted per field. The results was expressed in mean between the 10 slides The neonates were also analyzed in two groups based on umbilical artery Doppler status. At birth, the groups were compared for anemia and thrombocytopenia.
Results: The newborns with AEDV were delivered 4 weeks earlier and were smaller. AEDV neonates were significantly thrombocytopenic at birth as preterm born from preeclamptic mothers compared to control mothers newborns. Though annexin V was significantly higher in the control group, no significant correlations were found between its quantified assay and the hematologic variables of the mother and the newborn.
Conclusion: Hematologic status of the newborn at birth is proportional to the degree of placental dysfunction and is related to the fetal Doppler pattern. Although annexin V was higher in the control group, suggesting a role in the regulation of the coagulatory cascade, its clinical effects have to be defined.

214

Magnesium Sulfate Decreases Blood-Brain Barrier Permeability in Response to Acute Hypertension in Late-Pregnant Rats. Anna G Euser, Marilyn J Cipolla.* *Neurology and Ob/Gyn, University of Vermont, Burlington, VT, USA.*
Objective: Magnesium sulfate ($MgSO_4$) is widely used for prevention of eclampsia, despite an unclear mechanism of action. Eclampsia is a hypertensive disorder of pregnancy known to cause cerebral edema, most likely due to blood-brain barrier (BBB) disruption. Because $MgSO_4$ has been shown to be protective of the BBB in several brain injury models, we hypothesized that BBB protection may explain the effectiveness of $MgSO_4$ in eclamptic seizure prophylaxis.
Methods: *In vivo* BBB permeability to sodium fluorescein (NaFl) and Evan's blue (EB) was determined in late-pregnant (LP;d19-21) Sprague Dawley rats (n=11) after infusion of phenylephrine to raise blood pressure to cause autoregulatory breakthrough and compared to sham controls. A separate group of LP rats were treated with 270 mg/kg $MgSO_4$ i.p. every 4 hours for 24 hours prior to acute hypertension. Permeability in different brain regions (anterior, posterior and brainstem) was determined by infusion of EB and NaFl followed by flushing the vasculature with saline. The brains were homogenized and clearance of the dyes into the brain determined by fluorescence spectrophotometry, expressed as counts/sec/g brain tissue (CPS/g). Animals were ventilated to maintain blood gases within normal (PO_2>100mmHg, PCO_2=35-45mmHg).
Results: Permeability to NaFl was greater than EB in all groups, demonstrating size selectivity of the BBB ($p<0.05$). Acute hypertension caused a significant increase in EB permeability in all regions vs. sham; however, there was no significant increase in NaFl permeability. There was considerable regional heterogeneity in EB permeability such that the posterior region had the greatest increase in permeability (660%) followed by the anterior region (365%) and brainstem (170%); $p<0.05$ vs. sham. Treatment with $MgSO_4$ attenuated BBB permeability to EB after acute hypertension in the posterior region by 36% from 2958±469 vs.1852±385 CPS/g and by 60% in the anterior region from 1587±331 vs. 1165±258 CPS/g. There was no effect of treatment on permeability in the brainstem (n.s.).
Conclusions: These data demonstrate that acute hypertension causes increased BBB permeability in LP animals that was greatest in the posterior cerebrum, a region that is susceptible to edema formation during eclampsia. Treatment with $MgSO_4$ prior to acute hypertension attenuated BBB permeability, suggesting this may be one mechanism by which $MgSO_4$ prevents eclamptic seizures.

215

Maternal and Fetal Outcome after Heparin Intervention in Patients with Thrombophilia. Nazli Hossain,[1] Tahir Shamsi,[2] Nargis Soomro.[4] (SPON: Charles J Lockwood). *[1]Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, CT, USA; [2]Hematology, Bismallah Taqee Institute of Health Sciences, Karachi, Pakistan; [3]Department of Obstetrics and Gynecology Unit 2, Dow University of Health Sciences, Karachi, Pakistan.*
Objective: Heparin intervention is known to improve the live birth rates in women with known thrombophilia. This study was conducted to find out the maternal and fetal outcome in women with known thrombophilic disorders, after combined administration with low dose aspirin (LDA) and heparin.
Patients & Methods: Sixty five women with a history of at least 3 events of adverse pregnancy outcome were screened for both acquired and congenital thrombophilia at the Civil Hospital, University of Karachi, Pakistan. Screening included complete blood count, antiphospholipid antibodies IgG & IgM, and activities of protein C, protein S and antithrombin. All patients were evaluated for thrombophilia in the non-pregnant state. Pregnancy outcomes were compared between women receiving prophylactic heparin and low dose aspirin(study group) and the remaining women who did not receive treatment. (control group). The safety of heparin and LDA was also recorded.
Results: Of 65 women, with poor obstetrical history who were screened, 53(81%) women were found positive for either acquired or inherited thrombophilia. APLS was the most common cause seen in 30 (56%) while Protein C, S and antithrombin deficiency were seen in 13(24%), 8(15%) and 2(3.7%) women respectively. Sixteen women diagnosed as having thrombophilia (study group) received LDA and subcutaneous prophylactic heparin injection after viability scanning. In the study group, there were 13 (73%) live births as compared to only 2 (5.8%) in the control group. Miscarriages were seen in 3 (15.7%) women in study group as compared to 15 (44%) in control group. There was no fetal deaths in the study group as compared to 11 (32%) in control group. There was no case of heparin-induced thrombocytopenia or clinically significant bleeding. In 4 cases, self-limiting blood oozing from the site of injection was seen.
Conclusion: Patients with adverse pregnancy outcome should be screened for thrombophilia. LDA and heparin prophylaxis is associated with improved pregnancy outcome. It appears to be safe in pregnancy, but larger trials are needed to confirm efficacy and safety.

216

Basolateral Release of Thromboxane (TX), but Not Prostacyclin (PGI), by Placental Trophoblasts: Potential Role of TX-Induced Vasoconstriction in the Placental Vasculature. Shuang Zhao, Yanping Zhang, Yang Gu, David F Lewis, Yuping Wang.* *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*
Objective: The imbalance of increased TX and decreased PGI2 production by trophoblasts (TCs) contributes to the increased placental vasoconstriction in preeclampsia (PE). However, the mechanism of TC-released TX induced vasoconstriction in the placental vasculature is not clear. Because TCs are polarized epithelial cells, we determined if there are differential releases of TX and PGI2 by TCs towards apical and basolateral directions. Methods: TCs from normal placentas (n=7) were cultured in inserts with DMEM with 1.7ml in the upper chamber and 1.5ml in the lower chamber. After 48hrs of culture, the medium from the upper and the lower chambers were collected and measured for TX and PGI2 by their stable metabolites of TXB2 and 6-keto PGF1α,

respectively. To investigate if apical exposure of TCs to arachidonic acid (AA) and aspirin could affect TX and PGI production, AA+/-aspirin with different doses were added to the upper chamber and medium levels of TX and PGI2 were determined. Data are expressed as mean±SE and analyzed by ANOVA. A p level < 0.05 was set as statistically different. Results: 1) In control cells, TXB2 concentration was significantly higher in the lower than in the upper chamber, $p<0.05$; 2) TXB2 concentration was dose-dependently increased in cells treated with AA in both the upper ($p<0.01$) and the lower ($p<0.05$) chambers; 3) TXB2 concentrations were dose-dependently decreased in cells treated with aspirin+AA in both the upper and the lower chambers, but with a greater inhibitory effects in the upper chamber, $p<0.05$, respectively; and 4) There were no differences for 6-keto PGF1α levels in the upper and the lower chambers in control cells and in cells treated with AA +/- aspirin. Conclusions: 1) Basolateral release of TX, but not PGI2, is dominant in placental TCs; 2) Apical exposure of TCs to AA leads to a dose-dependent increase in TX production towards both the apical and basolateral directions without effect on PGI2 production. 3) Aspirin inhibits the AA-induced TX production to a greater degree to apical than to basolateral direction. These findings provide new insights into TC compartmental release of TX in the placenta, mechanisms of TX induced vasoconstriction in the placental vasculature, and possible explanation of the differential outcomes of aspirin in prevention of PE.

217

Adaptation to Sheer Stress in the Fetoplacental Vasculature of Human Term *In Vitro* Dually Perfused Placental Lobules from Pregnancies Complicated by Pre-Eclampsia and IUGR. Paul Brownbill, Colin Sibley.* *Division of Human Development, University of Manchester, Manchester, United Kingdom.*

Sheer stress is a haemodynamic property of blood flow which creates a biochemical signalling response in endothelia. In the fetoplacental vasculature of healthy placentas, sheer stress causes vasodilation through endothelial nitric oxide release. This study aimed to investigate the physiological adaptation to sheer stress in the *in vitro* dually perfused lobule of the term human placenta, to compare effects in four pregnancy groups: 1. healthy, 2. pre-eclampsia (PE), 3. IUGR, 4. PE with IUGR.

Placental lobules were perfused as previously described (Brownbill *et al*, 2006). Fetal and maternal inflows were incrementally and proportionately elevated (2.0 to 8.1 and 5.5 to 21.0 ml/min, respectively; groups 1-4: n=7, n=9, n=5 and n=4 lobules, respectively). Steady state fetal-side inflow hydrostatic pressure (FIHP) was recorded at each flow, as a measure of resistance, expressed as % Δ FIHP from that of the lowest inflow rate to standardise against variance in inherent resistance to flow between lobules, and then normalised, by logging, to permitting ANOVA between groups.

Each group assumed a hyperbolic profile when LOG % Δ FIHP (y-axis) was plotted against fetal-side inflow (x axis), with a diminishing curvature at higher flow. 2-way ANOVA of all groups showed a significant 'between groups' effect ($p < 0.0001$). Further analyses showed that groups 2 and 3 had similar adaptation profiles. Both group 2 and 3 showed a greater adaptive responses to flow at low to mid flow ranges when compared to group 1 ($p < 0.0001$, both). Group 4 had the poorest vasodilatory adaptation to flow, which was significantly different to all other groups ($p < 0.0001$, all comparisons) and was most noticeably different at the highest flow rates.

PE, or IUGR in isolation, showed a slightly greater *in vitro* adaptive response to flow at lower flow rates, compared to healthy lobules. When PE and IUGR coincided, the adaptive vasodilatory response to flow was almost absent. Whilst villous maldevelopment in IUGR may explain basal resistance to fetoplacental blood flow, our data has additionally highlighted an important physiological difference in adaptations to sheer stress between healthy and diseased pregnancies. We speculate that fetoplacental endothelial signaling is altered in PE, IUGR and PE with IUGR, possibly deriving different adaptive response phenotypes.

Brownbill *et al* (2006) Placenta 27, 560-7.

218

A Placental Perfusion Model To Investigate Irregular Placental Haemodynamics and the Release of Active Factors in the Pathophysiology of Pre-Eclampsia. Elizabeth S Hutchinson, Colin P Sibley,* Paul Brownbill, Philip N Baker,* Ian P Crocker. *Division of Human Development, University of Manchester, United Kingdom.*

Objective: Pre-eclampsia (PE) is characterized by maternal endothelial dysfunction, potentially initiated directly or indirectly by syncytiotrophoblast (ST) membrane fragments. It is possible that turbulent intervillous blood flow in PE, resulting from inadequate spiral artery adaptations, would liberate additional soluble non-particulate material which could also encourage vascular endothelial complications. We therefore used a placental perfusion system to replicate this process *in vitro*, and have investigated the effects of the soluble fractions on vascular endothelial cell cultures.

Methods: Placental lobules from term healthy human pregnancies (N=6) were dually perfused in open-circuit. The intervillous space was perfused at 14ml/min and then at 45ml/min, thus mimicking the flow turbulence and velocity of normal pregnancy and PE. Temporal perfusions were conducted at 14ml/min to permit histological comparisons. Collected maternal venous perfusates were centrifuged at 70,000g to remove particulate material. Generated soluble fractions were analyzed for the soluble markers of ST, lactate dehydrogenase (LDH), alkaline phosphatase (AP) and human chorionic gonadotrophin (hCG). The effect of these perfusion-generated soluble fractions on human umbilical vein endothelial cells (HUVECs) was determined by MTT assay.

Results: Low flow rate perfusions had minimal impact on villus tissue and ST. High flow rates caused ST vacuolation and shedding, at the points of cannulae insertion. At 14mls/min and 45mls/min the LDH levels in the maternal perfusates were 0.63±0.29u/ml/min and 1.15±0.47u/ml/min, respectively (ANOVA, $p<0.05$). There was an additional elevation under high flow in liberated AP and hCG. The soluble components from the high flow perfusions had a negative impact on endothelial cells in the MTT assay, reducing metabolic activity and viability of HUVECs by 44±6% at 6hrs incubation ($p<0.05$) and 48±9% at 24hrs ($p<0.05$), as compared to the low flow perfusates, independent of blood contamination.

Conclusions: These results provide evidence to confirm the hypothesis that turbulent intervillous haemodynamics could release material from the ST of a non-particulate, soluble nature. This material, like that of ST membrane fragments, may have the potential to initiate the vascular endothelial complications of PE *in vivo*.

219

Proteomic Analysis of the Endometrial Secretome. Jessica G Scotchie,[1] Marc A Fritz,*[1] Viorel Mocanu,[2] Mimi Mocanu,[2] Steven L Young.*[1] *[1]Obstetrics & Gynecology, University of North Carolina, Chapel Hill, NC, USA; [2]UNC-Duke Michael Hooker Proteomics Center, University of North Carolina, Chapel Hill, NC, USA.*

Objective: Understanding the changing endometrial secretome will allow further insight into endometrial function. Our objective is to characterize changes in the endometrial secretome during early and mid-secretory phases. **Methods:** Endometrial lavage was performed in 18-34 year old, normally cycling volunteers, 4 and 9 days after urinary LH surge. Proteins were concentrated and interfering substances removed using Ettan 2-D cleanup kit (GE). High-abundance proteins were depleted using Multiple Affinity Removal System (Agilent). LH+4 and LH+9 specimens were labeled with Cy3 and Cy5 fluorescent tags, respectively. The samples were combined and protein species separated using a 2D gel electrophoresis. Separate scanning for fluorescent signals corresponding to each tag allowed comparison of protein amounts using progenesis software. Spots were picked, in-gel digested with trypsin, eluted, and subjected to MALDI-TOF mass spectrometry for identification using GPS Explorer and Mascot software. **Results:** 14 proteins were identified as differentially expressed between days LH+4 and LH+9 as summarized in Table 1. Proteins showing increased expression from LH+4 to LH+9 have functions involving lipid transport, cell metabolism, stress response, coagulation, transcription regulation, phosphorylation, and immune modulation. Several proteins showing decreased expression from LH+4 to LH+9 had unknown functions; however, others were involved with immune modulation, host defense and lipid transport. One protein, apolipoprotein A1, was identified as having increased and decreased expression, suggesting altered post-translational modification causing differential gel migration. **Conclusions:** 2-D difference gel analysis can quantitate and identify proteins in uterine washings. Secretion of specific proteins is altered between the early and mid secretory phase.

Table 1. Differential Protein Expression Between LH+4 and LH+9

Degree Change	Increase	Decrease
Two-fold	Apolipoprotein A1	Alpha-1-β-glycoprotein
	Glutathione transferase	AF118063 (PRO1400)
	Heat shock protein 27	Transferrin precursor
		Sequence 105, Patent WO02094864 (unnamed protein product)
		IG lambda light chain
		Anti TNFα antibody
Three-fold		Hemoglobin-β variant
Four-fold	Fibrinogen gamma	
	Alpha enolase	
	Ig heavy gamma 1	
Five-fold	Secretory component/ Poly IgG receptor	Apolipoprotein A1

THURSDAY

220

Silencing of Natural Cytotoxicity Receptors at the Window of Implantation in Human Endometrium. Simcha Yagel,[*1] Irit Manaster,[2] Jacob Hanna,[2] Debra Goldman-Wohl,[1] Caryn Greenfield,[1] Shira Natanson-Yaron,[1] Yaron Hamani,[1] Yuval Bdolah,[1] Ronit Haimov-Kochman,[*1] Arye Hurwitz,[1] Ofer Mandelboim.[2] *[1]Obstetrics and Gynecology, Hadassah University Medical Center, Jerusalem, Israel; [2]The Lautenberg Center for Immunology, Hadassah University Medical Center, Jerusalem, Israel.*

Objective: We have recently demonstrated that NK (natural killer) cells play a critical role in trophoblast migration and angiogenesis at the fetal maternal interface. NK cells populate the endometrium at the secretory phase of the menstrual cycle, the time of anticipated blastocyst implantation. Peripheral blood (pb) NK cells and decidual NK cells (dNK) express a variety of activating receptors, including NKp44, NKp30 and NKp46-collectively known as natural cytotoxicity receptors (NCRs) and NKG2D which regulate NK cell killing and growth factor production. The aim of these experiments is to compare endometrial NK cell (eNK) activating receptor expression to pbNK and dNK cells and endometrial ligand expression with a focus on their role in blastocyst implantation.

Methods: Day 21 endometrium was collected from women with natural menstrual cycles. A lymphocyte profile of the endometrial cells and PB was performed. FACS analysis was performed on isolated endometrial NK cells and PB NK cells and dNK cells for CD56, CD16, NKp44, NKp30, NKp46 and NKG2D. NCR ligand expression was characterized on adherent endometrial cells using NCR-Ig fusion proteins and NKGD2-Ig and NKG2D specific ligands as well as control CCMI-Ig.

Results: Endometrial lymphocytes of day 21 are mostly CD56 bright CD16- NK cells, with T cells hardly observed, similar to dNK cells and in marked contrast to pbNK. Unlike pbNK and dNK cells, endometrial NK receptors do not express NKp30, NKp44 and NKG2D. CD56 is the only activating eNK receptor expressed. Interestingly, like decidual cells, adherent stromal endometrial cells expressed the ligands for NKp30, NKp44 and NKG2D suggesting that these NK cells have potential for activation.

Conclusion: These findings of a unique NK cell activating receptor profile on endometrial NK cells, unlike that of dNK, suggests a quiescent activation state between the NK cell and endometrial cells in preparation of an endometrial environment conducive for growth factor production and successful implantation of the hemiallogenic blastocyst.

221

Aberrant Endometrial Expression of Serum- and Glucocorticoid-Inducible Kinase-1 in Women with Unexplained Infertility. Fakhera Feroze-Zaidi, Julia Francis, Stephen J Smith,[*] Jan J Brosens. *Institute of Reproductive and Develomental Biology, Imperial College London, Hammersmith Campus, London, England, United Kingdom.*

Implantation is a complex process that requires coordinated interactions between the developing embryo and the constantly changing endometrium. High-density cDNA microarray analysis of timed endometrial samples (LH+5 to +10) was used to compare the transcript profiles of matched fertile women (n=8) and patients with unexplained infertility (n=6). Class Prediction Tool analysis identified 51 endometrial transcripts that were significantly differentially expressed between the fertile and infertile groups. Additional stringent statistical analysis as well as real-time quantitative PCR (RTQ-PCR) validation of a larger clinical sample set (fertile: n=20; infertile: n=15) confirmed that the expression of the serum- and glucocorticoid- inducible kinase-1 (SGK-1) mRNA was significantly higher in endometria of infertile women when compared to fertile controls. Laser capture micro-dissection followed by RTQ-PCR revealed SGK-1 transcripts were more abundantly expressed in surface epithelial cells than in glandular epithelial cells or in stromal cells. Furthermore, increased SGK-1 mRNA expression in infertile patients was confined to the surface epithelium and this was mimicked at protein level as determined by semi-quantitative immunohistochemical analysis. Treatment of endometrial explant cultures with either estrodial (E2, 10-8 mM), progesterone (P4, 10-6 mM), or a combination, revealed that SGK-1 mRNA expression is under progesterone control. In summary, SGK-1 is a serine/threonine kinase that critically regulates electrolyte transport over the cell membrane and is involved survival signalling. Its increased expression in endometrial surface epithelium of women with unexplained infertility suggests a possible mechanism of disrupting embryo apposition and implantation.

222

The Regulation of Leukemia Inhibitory Factor in First-Trimester Decidual Cells: Implication for the Pathogenesis of Preeclampsia. Chih-Feng F Yen,[1,2,3] Murat Basar,[1] Lynn Buchwalder,[1] William Murk,[1] Umit A Kayisli,[1] Aydin Arici,[*1] Frederick Schatz,[*1] Charles J Lockwood.[*1] *[1]Ob, Gyn & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Ob & Gyn, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, Taiwan; [3]Graduate Institute of Clinical Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan, Taiwan.*

Objective: Leukemia inhibitory factor (LIF), a member of the IL-6 cytokine family that exerts complex effects on inflammation, is critical for implantation, and is a molecular marker of endometrial blastocyst receptivity. Preeclampsia (PE) is associated with an exaggerated inflammatory response as well as restricted trophoblast invasion, which leads to impaired spiral artery remodeling. The pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β and thrombin are involved in various aspects of PE, suggesting that LIF may be modulated by these cytokines at the implantation site during placentation in preeclamptic decidua.

Methods: Human decidua from patients with PE and uncomplicated term deliveries (n = 4, respectively) were immunostained for LIF. Cultures of first-trimester decidual cells (FTDC) (n = 6) were primed with estradiol (E2) or with E2 and medroxyprogesterone acetate (MPA), and then treated with or without IL-1β, TNF-α, or thrombin. LIF levels in conditioned media were assessed by ELISA. LIF mRNA levels were measured by quantitative RT-PCR (qRT-PCR). Statistical analysis of the data was performed using Student's t-test and ANOVA followed by post hoc test.

Results: Immunostaining of LIF was significantly higher in decidual cells of patients with PE compared with normal specimens, with HSCORE value 175.0±11.9 and 106.7±9.5, respectively ($P<0.05$). ELISA showed no difference in FTDC-secreted LIF between E2 and E2+MPA treatments. IL-1β, TNF-α and thrombin significantly increased the secretion of LIF in FTDC (52.6± 12.0-, 14.1± 3.6- and 7.5± 3.3-fold, respectively; $P < 0.05$), and these effects were not altered by MPA. Corresponding effects on LIF mRNA levels were demonstrated by qRT-PCR.

Conclusions: Significant induction of LIF in FTDC by IL-1β, TNF-α and thrombin may be closely linked to the early pathogenesis of PE, as a compensatory mechanism in response to pro-inflammatory challenges during placentation.

223

Notch1 Signaling in the Endometrium Is Regulated by Human Chorionic Gonadotropin and Ovarian Steroids. Yalda Afshar,[1] Adina Stanculescu,[2] Lucio Miele,[2] Jaewook Jeong,[3] Franco DeMayo,[3] Asgerally Fazleabas.[*1] *[1]Dept of Ob/Gyn, University of Illinois, Chicago; COM, Chicago, IL; [2]Cardinal Bernadin Cancer Center, Loyola University, Maywood, IL; [3]Dept of Mol Cell Biology, Baylor College of Medicine, Houston, TX.*

In rodents and primates, human chorionic gonadotropin (hCG)-mediated inhibition of stromal cell apoptosis and their subsequent differentiation into decidual cells is critical for successful embryo implantation. A major regulator of cell survival and differentiation is the Notch1 receptor, which transduces extracellular signals responsible for cell fate determination during development. Proteolytic cleavage of full-length Notch1 (NFL) releases an active intracellular (NIC) peptide, which later translocates to the nucleus and activates gene transcription. A role of Notch 1 in decidualization response has yet to be determined.

Objective: To evaluate the role of Notch1 in the hCG-induced decidualization response.

Methods: Human uterine stromal fibroblasts (HuF) were decidualized with hCG (50IU), estrogen (E2; 36nM) and progesterone (MPA; 1μM) or vehicle, and levels of both Notch1 and its ligand, Jagged1, were determined by qRT-PCR, immunoblotting, and immunohistochemistry (IHC). Additionally, studies were extended to *in vivo* decidualization models. Specifically, we utilized a novel uterine-specific Notch1 knockout mouse model, and a simulated model of pregnancy in baboons.

Results: Significantly increased levels of NFL mRNA and protein were observed in decidualized HuF cells. E2 and MPA alone increased active NIC levels, whereas apoptosis (cytochalasin D; 10 μM) decreased NIC levels. Interestingly, hCG treatment partially rescued the cytoskeletal disruption. Pseudopregnant Notch1 knockout mice have reduced stroma, uterine weight, polyploidy and cyclin D3, all pointing towards a decidualization defect. A direct correlation was observed between Notch1 and hCG protein levels during pregnancy, suggesting that Notch1 regulates decidulization by preventing apoptosis. IHC analysis of cycling and stimulated pregnant baboon

endometrium demonstrate that while Jagged1 is constitutively expressed, Notch1 is induced by hCG; both proteins are expressed in luminal and glandular epithelium, as well as in the stroma.
Conclusions: The primate early embryonic signal, hCG, and Notch1 play critical roles during the window of uterine receptivity. We propose that Notch1 could be used as an early infertility marker. (Supported by HD 42280).

224

Maternal Cell Surface Changes at Implantation as Revealed by an In Vitro Model. H Singh,[1] L Nardo,[2] SJ Kimber,[3] JD Aplin.[1] (SPON: Philip Newton Baker). *[1]Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom; [2]Department of Reproductive Medicine, St Mary's Hospital, Manchester; [3]Faculty of Life Sciences, University of Manchester.*
The stages of embryo implantation have been defined based on morphological criteria but molecular mechanisms remain obscure. To overcome scarcity of material we have developed a model of implantation in which mouse embryos attach to human endometrial Ishikawa cells. These cells are polarized and express the surface mucin MUC1 which is associated with luminal epithelium in vivo. **Aims:**To quantify loose and stable embryo attachment in vitro. To examine the effect of embryo attachment on epithelial cell surface mucin distribution. To examine the effect of assisted hatching on embryo attachment. **Methods:**Blastocysts obtained from superovulated or naturally mated mice were collected at 96h post-hCG, and co-cultured in microwells with an Ishikawa cell monolayer in DMEM/FCS at 37 ^{0}C and 5% CO_2. Attachment was assessed by microscopic examination 48h after transfer. After PFA fixation, indirect immunofluorescence was performed with mouse mAb BC2 to detect MUC1 as a function of distance from the attachment site. **Results:**Blastocysts attached and outgrew efficiently (95%) on tissue culture plastic. Initially, blastocysts attached loosely to the cell monolayer at a high rate (85%). At 48h, 38% of blastocysts (n=413 total) had attached more stably to the epithelium and were retained after fixation. 57% of embryos undergoing assisted hatching attached (n=33 total). Ishikawa cells express MUC1 as a mosaic of varying intensity. 17% of blastocysts could modify the apical cell surface with loss of MUC1 from both the cell surface and intracellular locations, in patches stretching as far as approximately 50 cells from the embryo. This effect was not increased in embryos from assisted hatching. Normal levels of MUC-1 were retained on cells farther from the embryo. **Conclusions:**Initial weak attachment of a majority of embryos is followed by a phase in which a stronger adhesive interaction is developed by a smaller proportion of embryos. Loss of mucin occurs from surrounding epithelial cells. The mechanism of this process remains to be established. Attachment to Ishikawa cells is less efficient than on plastic and less efficient than in vivo in mouse, suggesting either selection by the maternal cell layer, or the need for embryo activation, or both. The model may be useful in discriminating embryos with varying implantation potential.

225

ERK1/2 MAPK Activity Is Involved in Human Endometrial Angiogenesis. William Murk, Serpil Uckac, Yesim H Uz, Hakan Cakmak, Umit A Kayisli, Aydin Arici.* *Dept. of OB, GYN & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objective: The extracellular signal-regulated kinase1/2 (ERK) subfamily is part of the Mitogen Activated Protein Kinase (MAPK) family of proteins and plays a role in a number of cellular processes such as cell proliferation and survival. ERK signaling is a noted regulator of angiogenesis, a critical, periodic process in the endometrium which reflects the cyclical growth and shedding of the menstrual cycle. We hypothesized that temporal changes in ERK phosphorylation (activity) occurs in human endometrial endothelial cells (HEEC) in parallel with angiogenic activity throughout the menstrual cycle and in early pregnancy.
Materials and methods: Staining for total- (T-) and phospho- (P-) ERK was performed on endometrial tissues (n=24) obtained from normal endometrium and decidual tissues (n=5) obtained from women with clinically normal pregnancies terminated voluntarily in the first trimester. Staining intensity was evaluated with HSCORE and grouped according to menstrual cycle phase. Endothelial cells isolated from normal endometrium were grown to confluence, and cultured cells were treated with or without specific ERK1/2 inhibitor [PD98059; 20 μM] for 48h. Cell proliferation was determined by MTS assay. Statistical analysis was done using one-way ANOVA, with $p<0.05$ considered significant.
Results: T-ERK staining was cytoplasmic and nuclear in the endothelial cells, and no significant change in expression was detected across the menstrual cycle, with strong staining throughout. P-ERK staining was nuclear, and showed the strongest immunoreactivity from the early to late proliferative phases (280.0±10.9 and 265.0±11.9, respectively), and underwent a gradual decrease from the early secretory phase (230.0±14.7) to a significantly decreased level in the late secretory phase (150.0±22.8, $p<0.001$). P-ERK staining in first trimester tissues was even further reduced (106.0±13.2, $p<0.001$). In vitro analysis revealed that inhibition of ERK signaling significantly reduced cell viability in endothelial cells compared to control ($p<0.01$).
Conclusion: Our results suggest that ERK activity is regulated in HEEC in a menstrual-cycle dependent manner. The highest level of ERK phosphorylation corresponds with the elevated angiogenesis in endometrium during the proliferative phase. Our in vitro data suggests that ERK activity is closely related with HEEC survival and supports our in vivo findings.

226

Suppression of Integrin-Linked Kinase Reduces Decidual Cell Survival and Adhesiveness to Extracellular Matrix. Umit A Kayisli,[1] Chih-Feng F Yen,[2] Sung H Kim,[3] Serpil Uckac,[1] Aydin Arici.*[1] *[1]Ob, Gyn & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Ob & Gyn, Chang Gung Memorial Hospital & University, Tao-Yuan, Taiwan, Taiwan; [3]Ob & Gyn, University of Ulsan College of Medicine, Seoul, Korea.*
Objective: Integrin-linked kinase (ILK) is a signaling protein that binds to the cytoplasmic domain of β1 integrin, and functions as a scaffold connecting integrins to the actin cytoskeleton and to its signaling pathways. Decidual cells produce a pericellular basal lamina (PBL) which consists of extracellular matrix components. Decidual cells bind to PBL via integrins. We have shown that ILK is involved in differentiation of stromal cells to decidual cells. We hypothesized that ILK plays roles in cell survival and integrin-mediated adhesion of decidual cells.
Materials and Methods: Immunohistochemistry with HSCORE was performed on the first-trimester tissues (n=5). Endometrial stromal cells (n=3) were cultured with E2 + medroxyprogesterone acetate (MPA) for in vitro decidualization, and then transfected with vehicle (control), ILK1 siRNA or fluorescent-conjugated (non-specific) siRNA. The efficacy of ILK siRNA transfection was examined in Western blotting. Quantitative in vitro adhesion (QA) assay was performed by counting the adherent cells to gelatin-coated culture plates at 30 and 60 min after seeding. Cell viability was evaluated with MTT cell proliferation assay in 96-well microplates. Statistical analysis was performed using Student's t-test and ANOVA followed by post hoc test.
Results: HSCORE revealed that ILK expression is markedly increased in decidualized stromal cells in comparison to the pre-decidual cells ($p<0.01$). Western blotting revealed siRNA knocked-down 40% of ILK protein in comparison to β-actin, while decidual cells treated with non-specific siRNA had no difference. The number of adherent cells was significantly decreased in the cells treated with ILK-siRNA (MEAN±SEM: 63.7±9.5 vs. 43.4±2.7, respectively; $P<0.05$). Neither adhesion nor the cell proliferation was changed in cells treated with non-specific siRNA. However, cell proliferation was significantly decreased in the cells treated with ILK-siRNA by MTT assay ($p<0.001$).
Conclusions: Increased ILK expression in decidual cells is involved in cell survival by up-regulating cell adhesion and proliferation, suggesting a role for ILK in transmitting signals from the pericellular basal lamina to intracellular signaling cascades.

227

Steroid Receptor Coactivator 2 (SRC-2) Is Essential for Implantation in the Mouse: Translation to the Human. Paula Amato,[1] Atish Mukherfee,[2] Rodrigo Fernandez-Valdivia,[2] Jaewook Joeng,[2] Franco J DeMayo,[2] Sandra A Carson,*[1] Bert W O'Malley,[2] John P Lydon.[2] *[1]Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Molecular & Cell Biology, Baylor College of Medicine, Houston, TX, USA.*
Objective: To investigate the role of SRC-2 in progesterone receptor (PR)-mediated physiological processes required for the maintenance of fertility, a novel PRCre/+SRC-2flox/flox bigenic mouse was generated [1] to ablate SRC-2 function specifically in cell lineages that express PR. Ablation of SRC-2 resulted in an infertility phenotype in the female. The PRCre/+SRC-2flox/flox infertility defect is due to uterine implantation failure, underscoring the unique importance of SRC-2 in peri-implantation biological processes that require progesterone signaling. The purpose of this study was to investigate the expression and functional significance of SRC-2 in the human endometrium.
Methods: Endometrial biopsy samples were obtained using an endometrial Pipelle from regularly cycling women, aged 18-35 years, during the late proliferative phase (cycle day 10-14), mid-luteal (cycle day 20-24) and late

luteal phase (cycle day 24-28) of the menstrual cycle. Immunohistochemical detection of human SRC-1, SRC-2, SRC-3, PR and ER, and real-time PCR analyses and in-situ hybridization were performed according to procedures described previously[1].
Results: While real-time PCR demonstrated SRC-2 mRNA levels were highest during the late-proliferative/early luteal phase of the menstrual cycle, in-situ hybridization and immunohistochemistry revealed uterine PR and SRC-2 localize to identical cell-lineages in the glandular epithelium and stroma of the human endometrium, with prominent expression of both proteins at the mid-luteal phase of the cycle.
Conclusions: As observed recently in the mouse, the spatiotemporal expression profiles of PR and SRC-2 can be superimposed in the human endometrium. These data provide supportive evidence for an essential role for SRC-2 in progesterone-initiated uterine functions in humans. Ongoing in vitro studies are investigating the functional significance of SRC-2 in cultured human endometrial stromal cells and its role in decidualization.
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228

Expression of Intermedin, a Novel CT/CGRP Family Peptide in Implantation Sites: Infusion of IMD_{17-47} Restricts the Growth of Rat Implantation Sites. Madhu S Chauhan, Luckey C Reed, Uma Yallampalli, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Intermedin (IMD)/Adrenomedullin (AM) 2 is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) family. Recently we demonstrated that IMD antagonist, IMD_{17-47} causes feto-placental growth restriction, apoptotic changes and impairment of placental morphology. However, it remains to be assessed if IMD transcript and protein are expressed early on in rat implantation sites. Based on our studies in rat placenta, we hypothesize that IMD mRNA, as well as protein, are expressed in the implantation site and may be involved in embryo implantation and decidualization in rats.
OBJECTIVES: 1) To assess the expression of the mRNA transcript and immunolocalize IMD protein in rat implantation sites; 2) To assess the effects of IMD_{17-47} infusion on the weights and numbers of implantation sites; and 3) to assess the expression of pro-apoptotic proteins, caspase 3 and cytochrome c in implantation sites of IMD_{17-47} treated rats.
METHODS: Sprague Dawley rats were used in this study. Osmotic mini-pumps containing vehicle alone or IMD_{17-47} (200 μg/day) were inserted s.c. in pregnant rats on Day (D) 3 of gestation and sacrificed on D9 (n = 5). Total number and weights of the implantation sites were recorded. Total RNA and protein was isolated from implantation sites using TRIzol reagent and processed for RT-PCR and Western blots. The results are expressed relative to 18S mRNA or β-Tubulin.
RESULTS: Our data demonstrates that: 1) IMD transcript is expressed in rat implantation sites; 2) IMD protein is expressed and localized in labyrinth zone of giant trophoblast cells of D9 rat implantation site; 3) IMD_{17-47} causes a significant decline in the weights of D9 implantation sites ($p<0.05$) but has no effect on their number; and 4) IMD_{17-47} has no effect on the expression of pro-apoptotic proteins caspase 3 and cytochrome C in implantation site.
CONCLUSION: IMD is expressed in implantation sites on D9 of pregnant rats. Inhibition of IMD effects caused a decrease in the weights of implantation sites suggesting a potential role for endogenous IMD during post implantation period in rat pregnancy. Further, in contrast to the effects observed in D18 rat placenta, these effects are not mediated through apoptosis in implantation sites suggesting that: IMD effects may be gestational age and tissue specific.

229

TGF-Beta 3 Regulates Mediators of Uterine Receptivity in Human Endometrial Stromal Cells. Erin F Wolff, Jason G Bromer, Hongling Du, Hugh S Taylor.* *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Introduction:
HOXA10 and HOXA11 are known to be expressed in human endometrium. Targeted disruption of either of these two gene products is known to result in infertility due to a specific defect in implantation,demonstrating that these genes are important for endometrial receptivity. Transforming Growth Factor Beta 3 (TGF-β3) has been shown to be expressed in the endometrium. It has previously been suggested that during the peri-implantation period, the TGF-beta signaling pathway plays an important role in endometrial receptivity and embryo attachment to sub-epithelial stroma. S100A14 has been also demonstrated to be a target of HOXA10 regulation in human endometrium. We hypothesized that TGF-β3 may act through these important transcriptional regulators of endometrial receptivity.
Materials and Methods:
Human endometrial stromal cells were grown to 70% confluence in DMEM with 10% FBS and 10% antibiotic/antimycotic. Media was then changed to steroid free, phenol red free DMEM with 10% charcoal stripped FBS for 12 hrs. Cells were subsequently cultured with recombinant human TGF-β3 protein in concentrations of 0 (control), 0.01, 0.1, 1, 5, and 10 ng/ml for 24 hours. Cells were harvested and mRNA was extracted using the RNAeasy method. cDNA was generated using iScript, and quantitative real time RT-PCR using SybrGreen was performed and normalized to β-actin. Fold change in normalized Hoxa10, Hoxa11, and S100A14 expression was assessed. All experiments were conducted in triplicate, repeated four times and compared using ANOVA.
Results:
After incubation with TGF-β3 for 24 hours, a dose-dependent response in the mRNA expression of all three gene targets was observed. HOXA10 demonstrated a change in fold expression from 0.99 at a concentration of 0.01 ng/ml to 2.84 at 10 ng/ml. HOXA11 showed an initial decrease of 0.87 fold at 0.01 ng/ml, and a maximum increase of 3.37 fold at 10 ng/ml. S100A14 also showed an initial decrease of 0.76 fold at 0.01 ng/ml, and demonstrated the largest fold increase in expression of 11.27 at 10 ng/ml.
Conclusions:
In vitro, treatment with TGF-β3 induced a dose-dependent change in expression of HOXA10, HOXA11. Furthermore, TGF-β3 also induced a dose response in the known HOXA10 target gene, S100A14. TGF-β3 appears to play a role in the regulation of known genes necessary for endometrial receptivity.

230

A Novel Three-Dimensional In Vitro Implantation Model To Study Primate Embryo Development and Early Pregnancy. Tien-cheng Chang,[1,2,3] Gennadiy I Bondarenko,[2,3] Behzad Gerami-Naini,[1,2,3] Jessica G Drenzek,[1,2,3] Maureen Durning,[2,3] Mark A Garthwaite,[2,3] Thaddeus G Golos.[1,2,3] (SPON: Ronald R Magness). *[1]Endocrinology-Reproductive Physiology Program, University of Wisconsin-Madison; [2]Department of Obstetrics and Gynecology, University of Wisconsin Medical School, Madison, WI, USA; [3]Wisconsin National Primate Research Center, Madison, WI, USA.*
OBJECTIVE: To develop a model to study implantation and placenta formation in vitro with rhesus monkey embryos.
HYPOTHESIS: A novel nonhuman primate in vitro 3-D system can provide cues for implantation and interaction with the extracellular environment not available in 2-D planar models.
METHODS: We developed an in vitro 3-D implantation model utilizing blastocyst stage rhesus monkey embryos embedded in 3-D Matrigel explants, coupled with different feeder cell microenvironments.
RESULTS: Signs of implantation including enlargement of embryo mass, invasion and proliferation of trophectoderm cell layers, cystic formation, and cellular outgrowths derived from the embryo, initiated within the first week post embedding. Trophoblast structures with protrusion and branches growing from the surface of embryo implants were observed. Rapid proliferation and differentiation of the trophoblast structures provided evidence of interactions between the embryo and the 3-D environment up to 45 days in culture. Immunohistochemical staining for CG and other biomarkers, combined with immunoassays for CG and progesterone showed secretion curves similar to early pregnancy, indicated positive characteristics of trophoblastic cell lineages. In addition, our study found morphological factors to predict successful establishment and prolonged embryo development, as well as an optimized culture microenvironment of media and feeder cells.
CONCLUSIONS: We have established a 3-D in vitro system showing the potential to model implantation initiation in vitro, and revealed the capability of the embryo to interact with the extracellular matrix. Continuing studies will accelerate our understanding of nonhuman primate embryo development, with potential for insights into early pregnancy loss and related pathologies.

231

Endometrial Nucleolin Shows Specific Patterns of Aberrant Expression in Different Types of Recurrent Pregnancy Loss. Dharani K Hapangama,[1] Jo Drury,[1] Carmen M Martin-Ruiz,[2] Thomas Von Zglinicki,[2] Siobhan M Quenby.*[1] [1]*School of Reproductive & Developmental Medicine, University of Liverpool, Liverpool Women's Hospital, Liverpool, United Kingdom;* [2]*Henry Wellcome Lab. for Biogerontology Research, University of Newcastle Newcastle General Hospital, Newcastle upon Tyne, United Kingdom.*

Aims / Objectives: In order to assess whether markers of cell senescence are related to Recurrent Miscarriage (RM), we assessed the expression of nucleolin in endometrial biopsies from women with RM.

Materials and methods: This prospective pilot study included 49 women of whom, 10 had idiopathic recurrent fetal loss (miscarriage following identification of fetal cardiac activity) (Group 1), 10 had idiopathic recurrent loss of empty gestation sacs (Group 2), 10 had recurrent implantation failure (Group 3) and 19 had two or more normal pregnancies(Group 4). An endometrial sample was collected during the implantation window (cycle day 22+/-2) from each woman and dated according to recent modifications of Noyes criteria by two experienced pathologists1. Nucleolin expression was evaluated by immuno-histochemistry using a commercially available monoclonal antibody and a semi-quantitative scoring system. We also correlated this with the mean endometrial cell telomere length and endometrial telomerase expression.

Results: The endometria of fertile, healthy women showed virtually no nucleolin immuno-reactivity during the implantation window. However, in Group 1, immuno-staining for nucleolin was significantly increased in glands and luminal epithelium ($p =<0.001$). The perivascular nucleolin expression was significantly raised in groups 2 & 3 ($p<0.05$). There were no significant differences in mean endometrial TL between groups. However, the nucleolin expression was closely related to the endometrial telomerase expression.

Conclusions: These data provide novel insight into the biological correlates of clinical types of RM and suggest that specific alterations in the regulation of endometrial cell fate are associated with different types of recurrent pregnancy loss.

232

Oestrogen and Progesterone Differentially Regulates VEGF mRNA Expression in the Mouse Uterus. Lisa M Walter, Peter AW Rogers,* Jane E Girling.* *Centre for Women's Health Research, Monash University Dept. OBGYN and Monash Institute of Medical Research, Melbourne, VIC, Australia.*

Objective: Appropriate and tightly controlled changes in the vasculature are essential for proper functioning of the endometrium during the menstrual cycle. Although these endometrial vascular changes are controlled by oestrogen (E) and progesterone (P) and mediated in part by the angiogenic protein vegf, the molecular mechanisms underlying the process are not well understood. In this study we quantified the relative changes in mRNA expression of total vegf, individual vegf isoforms (120, 164, 188) and the vegf receptors (flk-1, nrp-1, nrp-2) in uteri from pregnant and hormone-treated mice. We hypothesised that expression of the isoforms and receptors would increase concurrent with the endometrial angiogenesis that is known to occur during early pregnancy and following E and P treatment in the mouse.

Methods: *Pregnancy*: Uteri were collected from mice (CBA x C57) on days 1-5 of pregnancy (n=6 per day). *P Regime*: Mice (n=8) were given a single injection of 100 ng of estradiol on day 8 following ovariectomy, followed by a day with no treatment and 3 consecutive daily injections of 1 mg P. Other groups were treated with either the vehicle (n=7) or P (n=8) only. All mice were dissected on day 13 after ovariectomy. *Short-term E Regime*: Mice (n=7) were given a single injection of 100 ng of estradiol and dissected 24 hours later. mRNA expression was quantified by real time RT-PCR and normalized against 18S rRNA.

Results: Overall, the mRNA expression levels were of an equivalent range in pregnant and E-treated animals, but considerably lower than those in vehicle and P-treated animals. Expression of total vegf, $vegf_{120}$, $vegf_{164}$, flk-1, nrp-1 and nrp-2 mRNA increased significantly across early pregnancy, correlating with the endothelial cell proliferation that also occurs at this time. In contrast, levels of total vegf, $vegf_{120}$, $vegf_{188}$, flk-1, nrp-1 and nrp-2 mRNA were significantly lower in animals dissected 24 hours following E treatment compared to those treated with vehicle or P, despite endometrial angiogenesis occurring in response to this hormone.

Conclusions: Although E has pro-angiogenic effects in the endometrium, we conclude that E down-regulates mRNA expression of vegf isoforms and receptors in the mouse uterus. Basal levels of E may be responsible for the lower overall mRNA expression levels observed in pregnant mice in contrast to those seen in P or vehicle-treated mice.

233

Pattern of Expression of Notch Protein in Normal and Pathological Endometrium. Luigi Cobellis,*[1] Maria De Falco,[2] Annunziata Mastrogiacomo,[1] Gabriele Coppola,[3] Antonio De Luca,[3] Francesca Caprio,[1] Maria Teresa Schettino,[1] Nicola Colacurci.[1] (SPON: Felice Petraglia). [1]*Dept. of Obstetrics and Gynecology, II University of Naples, Naples, Italy;* [2]*Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, Federico II University, Naples, Italy;* [3]*Dept. of Medicine and Public Health, Section of Clinical Anatomy, II University of Naples, Naples, Italy.*

Hypothesis. Human endometrium, during every reproductive cycle, undergoes extensive tissue remodelling in response to cyclic hormonal changes. Angiogenesis,plays an important role in the remodelling of endometrial tissue. Notch receptors and their ligands have an important role in cell specification, proliferation and apoptosis,but also in the regulation of vascular formation.In this study was investigated the cellular localization of Notch-1,Notch-4 and Jagged-1 in normal and pathologic endometrium.Notch activation can modulate cell cycle regulation,so we evaluated the expression of cyclin D1 and p21. **Methods.**60 samples of physiologic human endometrium (20 in proliferative,20 in secretive and 20 in menopause) and 60 samples of pathologic human endometrium (20 hyperplasia,20 post-menopausal polyps and 20 cancer) were immediately fixed in formalin for ihc.**Results.** Notch-1 and Jagged-1 showed an increase from proliferative to secretive phase,instead Notch-4 decreased from proliferative to secretive phase.In contrast all three of them were expressed almost to undetectable level in menopause endometrial tissue.In pathologic endometrium Notch-1 showed a weak expression in polyps that becomes almost undetectable in the hyperplasia and carcinoma.Notch-4 and Jagged-1 was well expressed in the polyps but lower in the hyperplasia and carcinoma. Finally in physiological conditions cyclin D1 is intensely expressed during the proliferative phase but decreased in the secretive phase and menopause.p21 showed a weak level during the proliferative phase,a moderate level during the secretive phase and undetectable level in menopause.In pathological conditions cyclin D1was strongly expressed in polyp and in carcinoma,moderately lower in hyperplasia.The expression of p21was undetectable in polyp,weak in carcinoma and strongly increased in hyperplasia section.**Conclusion**We have characterized the expression pattern of Notch receptors and their ligands during the tissue remodelling of human endometrium in physiologic conditions,and compared the Notch pathways in several patological endometrial conditions.

234

Parathyroid Hormone-Like Hormone Mediates the Estradiol-Induced Increase in Osteopontin in Ishikawa Cells. Chandrasekhar Thota, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

INTRODUCTION: Osteopontin (OPN) reported in glandular epithelial cells in human endometrium, and in secretions of uterus was reported to play a role in the process of decidualization. Parathyroid hormone-like hormone (PTHLH) reported in endometrium at the site of implantation was also reported to increase OPN in the kidney suggesting that OPN may be mediating the PTHLH effects.

OBJECTIVES: In this study we used Ishikawa cells to: 1) assess the expression of OPN in response to PTHLH and regulation of OPN by steroid hormones; and 2) assess if PTHLH mediates steroid hormone effects on OPN

METHODS: Ishikawa cells cultured in MEM at 37°C and 5% CO_2 were treated with $PTHLH_{1-34}$ (1, 10, 100 and 1000nM) and PTHLH antagonist, $PTHLH_{7-34}$ (100nM) either alone or in the presence of $PTHLH_{1-34}$ (100 nM). To study the steroid hormone regulation of OPN, Ishikawa cells were treated with 1, 10, 100nM concentrations of estradiol or progesterone, and to assess if PTHLH mediates steroid hormone affects, the cells were treated with estradiol (100nM) and progesterone (10nM) in the presence of $PTHLH_{7-34}$ (100 nM). Total RNA and protein isolated were subjected to RT-PCR and western analysis for OPN expression using specific primers and antibody, respectively.

RESULTS: RT-PCR analysis of total RNA demonstrated abundant expression of OPN in Ishikawa cells. OPN mRNA was increased in cells treated with 10 and 100 nM of $PTHLH_{1-34}$, and with all doses of estradiol-17b. Increases observed in LIF mRNA in progesterone treated cells were not significant. Expression of OPN protein was higher at 10 and 100nM of $PTHLH_{1-34}$, 1 and 10nM of estradiol and 10, and 100nM of progesterone. OPN mRNA and protein levels decreased in cells treated with $PTHLH_{7-34}$ both in the presence and absence of estradiol. However, no significant differences were observed in the presence of progesterone. $PTHLH_{1-34}$ effectively reversed the $PTHLH_{7-34}$ induced decreases in OPN mRNA and protein.

CONCLUSION: Our results suggest that in Ishikawa cells $PTHLH_{1-34}$ and estradiol upregulate while PTHLH antagonist $PTHLH_{7-34}$ downregulate OPN mRNA and protein expression. Effects of progesterone are not significant. Our results also suggests that increases in OPN expression observed in estradiol-treated cells were reversed by $PTHLH_{7-34}$ suggesting that PTHLH mediates the estradiol induced increases in OPN in Ishikawa cells.

235

Development of High Throughput Analysis of Endometrial Biopsies. Richard E Leach,[*5] Randall Armant,[*1] Ruba Ali-Fehri,[1] Christos Coutifaris,[*2] Linda Guidice,[3] Phyllis Leppert,[*4] Michael Diamond,[*1] NICHD Reproductive Medicine Network. *[1]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI; [2]Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA; [3]Department of Obstetrics and Gynecology, University of California San Francisco, San Francisco, CA; [4]Department of Obstetrics and Gynecology, Duke University, Durham, NC; [5]Department of Obstetrics and Gynecology, University of Illinois, Chicago, IL.*

Objective: The timed endometrial biopsy followed by histological dating of the endometrium provides no clinically useful information as a screening test for infertile women (*Fertil Steril* 82, 1264-72, 2004). Heparin-binding EGF-like growth factor (HB-EGF) is increased in the endometrium during the secretory phase in humans. The addition of recombinant HB-EGF to human blastocysts significantly increases trophoblast differentiation in vitro. These important biological actions led us to test HB-EGF protein accumulation in endometrial biopsies for its capacity to differentiate between fertile and infertile women recruited by the NICHD Reproductive Medicine network.

Methods: Endometrial biopsies from patients (n=173) originally collected by the Reproductive Medicine Network were biopsied on cycle days 21-22 and cycle days 26-27 respectively. Fertile and infertile women were equally distributed between the 2 biopsy intervals. Paraffin embedded specimens were immunostained for HB-EG using a robotic DAKO Autostainer. To visualize and quantify (grey level) antigen, a peroxidase kit DAKO was used in conjunction with image analysis, according to our published procedure (*Lancet* 360, 1215-19; 2002). ANOVA was used for statistical analysis with significance at * $p<0.05$.

Results: When biopsy specimens are segregated into early, mid and late secretory phase based on histological dating there is a significant increase in HB-EGF grey scale level in both the luminal and glandular epithelium in the mid and late secretory phase. There was no difference in protein accumulation in the endometrial stroma across the secretory phase. There was no difference in HB-EGF levels between fertile or infertile women.

Conclusions: This is the first high throughput analysis of a protein marker of the implantation window in well characterized endometrial biopsies. This proof of concept will enable us to investigate other proteins with known function and novel genes currently being identified by gene array analysis.

236

Parathyroid Hormone-Like Hormone Mediates the Estradiol-Induced Increase in Leukemia Inhibitory Factor in Ishikawa Cells. Chandrasekhar Thota, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

INTRODUCTION: Leukemia inhibitory factor (LIF) is induced in stromal cells surrounding active blastocyst at the time of attachment reaction and is important for implantation. Parathyroid hormone-like hormone was reported to increase LIF in osteoblasts suggesting a regulatory role for PTHLH in LIF expression.

OBJECTIVES: In this study, we used Ishikawa cells, a human endometrial adenocarcinoma cell line to: 1) assess the expression of LIF in response to PTHLH and regulation of LIF by steroid hormones; and 2) assess if PTHLH mediates steroid hormone effects on LIF.

METHODS: Ishikawa cells cultured in MEM at 37°C and 5% CO_2 were treated with $PTHLH_{1-34}$ (1, 10, 100 and 1000nM) and PTHLH antagonist, $PTHLH_{7-34}$ (100nM) either alone or in the presence of $PTHLH_{1-34}$ (100 nM). To study the steroid hormone regulation of LIF, Ishikawa cells were treated with 1, 10, 100nM concentrations of estradiol or progesterone, and to assess if PTHLH mediates steroid hormone affects the cells were treated with estradiol (100nM) or progesterone (10nM) in the presence of $PTHLH_{7-34}$ (100 nM). Total RNA and protein were subjected to RT-PCR and western analysis for LIF expression using specific primers and antibody, respectively.

RESULTS: RT-PCR analysis demonstrated expression of LIF in Ishikawa cells. $PTHLH_{1-34}$ treatment caused increases in LIF mRNA expression in three (10, 100 1000 nM) of the four doses tested. Estradiol-17b increased LIF mRNA expression at 1, 10 and 100 nM while progesterone increased LIF mRNA at 10 nM concentration. LIF mRNA showed a decrease in cells treated with $PTHLH_{7-34}$ both in the presence and absence of estradiol. However, no significant differences were observed in cells treated with progesterone. $PTHLH_{1-34}$ reversed the $PTHLH_{7-34}$ induced decreases in LIF mRNA and protein in Ishikawa cells. Expression of LIF protein was higher at 100 nM of $PTHLH_{1-34}$, 1, 10 100 nM estradiol and 10 and 100 nM of progesterone.

CONCLUSION: Our results suggest that in Ishikawa cells $PTHLH_{1-34}$ and estradiol upregulate while PTHLH antagonist $PTHLH_{7-34}$ downregulates LIF mRNA and protein expression. Progesterone effects on LIF expression appears to be inconsistent. Our results also suggests that increases observed in LIF expression in estradiol-treated cells were reversed by PTHLH antagonist $PTHLH_{7-34}$ indicating that PTHLH may mediate the estradiol-induced increases in LIF in Ishikawa cells.

237

Estrogen Is Not Essential for Full Endometrial Restoration Following Endometrial Shedding in a Mouse Model. Tu'uhevaha Kaitu'u-Lino,[1,2] Naomi Morison,[1] Lois Salamonsen.[1] (SPON: Caroline E Gargett). *[1]Prince Henry's Institute; [2]Dept Ob-Gyn, Monash University, Melbourne, Victoria, Australia.*

Dogma regarding endometrial regeneration following menses is that estrogen (E)-primed proliferation is required for re-establishment of full thickness endometrium and that while it may not be required for initial re-epithelialization of the uterine surface, it is essential for stromal renewal. Lack of suitable animal models has limited functional studies on menstruation and endometrial repair.

Objective: To determine if E is essential for endometrial restoration in a mouse model.

Rationale: In the model[1,2] one uterine horn of each ovariectomised (ovx) mouse is decidualized and progesterone (P) support withdrawn. Endometrial breakdown is generally complete by 24h and repair by 48h, closely resembling events in the human endometrium at menses. In this model, which lacks ovarian E, the endometrium is rapidly and fully restored. However, estrogenic influences from extra-ovarian sources (diet and fat) remain: dietary E has significant effects on uterine weight[3].

Methods: Two groups of mice were used: control (n=13); ovx + normal diet + vehicle, and E-free (n= 16); ovx + soy-free diet + letrozole (20mg/day after P withdrawal). At 48h uterine weights were measured, uterine mRNA (selected animals) was subjected to qRT-PCR for E responsive genes lactoferrin and progesterone receptor (PR). Analysis of genes included an E-replaced group. Differences in endometrial restoration were assessed using a previously developed morphological scoring system[2].

Results: Uterine weight did not differ between control and E-free groups (15.5±3.4mg vs 14.9±2.1mg). For lactoferrin mRNA (corrected for 18s), control (1.02±0.75 relative units) was not different to E-free (3.1±2.9), but E-replaced was significantly higher (176.8±190.6, $p\leq0.05$) with similar findings for PR mRNA (6.8±5.9 vs 6.0±3.6 vs 87.2±60.7 relative units, $p\leq0.05$), suggesting that extra-ovarian estrogenic influences are minimal after ovariectomy. Importantly, scoring of uterine morphology showed no difference in endometrial restoration; endometrium (epithelial and stromal components) from both groups restored to a normal phenotype within 48h of P withdrawal.

Conclusion: E is not required for successful endometrial restoration in this model, suggesting it may also not be essential for re-establishment of endometrium in women.

[1]Brasted et al 2003 Biol Reprod 69:1273, [2]Kaitu'u-Lino et al 2006 Cell Tiss Res (in press), [3]Britt et al 2005 Menopause 12:l74.

238

Endometrial Regulation by Epithelial Membrane Protein-2. Madhuri Wadehra, Jonathan Braun, Andrea Niklaus. (SPON: Gautam Chaudhuri). *Pathology and Laboratory Medicine, Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Introduction

The identification of molecules required for implantation is an active area of research in contraceptive and infertility biology. Epithelial membrane protein-2 (EMP2), a member of the tetraspan superfamily, has emerged as a regulator of endometrial function. Specifically, EMP2 expression, primarily expressed in the luminal epithelium of the endometrium, is necessary for successful uterine receptivity, and dysregulation of its expression has been shown to correlate with cancer progression. By combining LCM and microarray technology to investigate the molecular phenotype of epithelial specific cells, we identified

biological networks of proteins and specific gene function families related to EMP-2, that were significantly regulated by ovarian hormones in the endometrium of women of mid –reproductive age. Examination of these networks has revealed an important role for EMP2 in guiding the delivery of a distinct network of adhesion proteins related to the integrin mediated pathway to distinct domains on the plasma membrane.

Results:

Previous experiments reveal the requirement of EMP2 expression for successful implantation as well as in cancer prognosis. We have previously shown that EMP2 is regulated by progesterone, and that its expression regulates key integrin pairs such as αvβ3 integrin. LCM microarray technology was employed to identify epithelial-specific candidate proteins that may be regulated by EMP2. Ease analysis revealed significant epithelial over representation of more than 12 members of the integrin mediated pathway. Thus far, LCM microarray analysis has revealed at least 4 candidate proteins that are regulated by EMP2. These include MUC-1, MUC-4, OSF-2, osteopontin, and HEF-1. This important molecular signature is critically for understanding the normal biologic and pathophysiologic functions of EMP2.

Conclusion:

This study demonstrates that EMP2 expression helps control the surface expression of key adhesion interacting proteins. To our knowledge, this is the first example of such regulation of a membrane protein's trafficking itinerary in the endometrium. Given the role of EMP2 in implantation and in cancer progression, we predict that this type of molecular modeling will allow for novel therapeutics in contraception design as well as in cancer treatment.

239

Quantitative Cellular and Molecular Analysis of the Effect of Progesterone Withdrawal in a Murine Model of Decidualization. Ching-wen Cheng,[1,2] Holli Bielby,[1,2] Di Licence,[1,2] Stephen K Smith,[*2,3] Cristin G Print,[1,4] David Stephen Charnock-Jones.[*1,2] *[1]Department of Pathology, University of Cambridge, United Kingdom; [2]Department of Obstetrics and Gynaecology, University of Cambridge, United Kingdom; [3]Imperial College, London, United Kingdom; [4]University of Auckland, Auckland, New Zealand.*

Objective: To investigate the dynamic changes and the underlying mechanisms that occur in human endometrium using a murine model which mimics decidualization and regression.

Method: Ovariectomized mice were treated sequentially with steroid hormones and then, to induce decidualization, oil was injected into the uterine lumen. Animals were then divided into two groups in which progesterone treatment was maintained or progesterone treatment was withdrawn. The uterine tissues were collected at several time-points after the induction of decidualization. Immunohistochemical, morphometric and microarray experiments were used to study the cellular and molecular changes that accompany decidualization and tissue regression.

Result: Decidualization occurred in all experimental animals, and a process similar to menstruation was observed following this in the progesterone-withdrawal group. Histological analysis demonstrated that the decidualization and subsequent tissue degeneration observed in this model shared features with human decidualization and menstruation, including oedema and haemorrhage. The volume fractions of leukocytes, macrophages and neutrophils, but not endothelial cells, increased in decidualized uterus and decreased after progesterone withdrawal-induced tissue degradation was completed. The microarray data showed that the levels of many RNA transcripts encoding immune-related factors changed during the time-course studied. These included transcripts encoding chemokines, cytokines and acute phase proteins. The transcript levels of many of these factors paralleled the changes in the volume fraction of the immune cells. Additional transcripts known to be regulated in human endometrium are also regulated, for example, glutathione peroxidases, metallothioneins and glutathione S-transferases.

Conclusion: We have used a murine model of human endometrial decidualization and regression to show that immune cells are recruited into menstruating endometrium, and immune-related genes are regulated in uterus during endometrium remodelling. This model provides a useful alternative to non-human primates with the advantage that it is amenable to genetic manipulation.

240

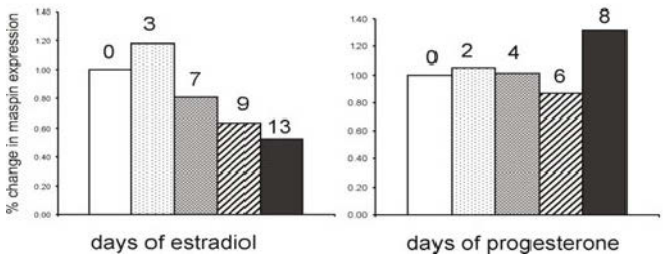
Estradiol Down-Regulates Maspin Expression in Human ECC-1 Cells. Christopher S Sipe, Jeremy E Coffin, Anuja Dokras.[*] *Reproductive Endocrinology & Infertility, University of Iowa Hospitals & Clinics, Iowa City, IA, USA.*

Objective: The mammary serine protease inhibitor, maspin, is expressed in several human tissues and has been shown to play a role in placental invasion. In the human endometrium maspin is primarily expressed in the glands. Previously, we have demonstrated that maspin expression doubles in the secretory phase compared to the proliferative phase. The objective of this study was to investigate whether maspin expression may be regulated by estradiol and progesterone in human endometrial cells.

Methods: We used a well differentiated human endometrial epithelial cancer cell line (ECC-1) grown in a 1:1 mixture of DMEM:Hams F-12 with 10% FBS and 0.1% gentamycin. Cells were cultured with and without 1μM 17β-estradiol, and harvested at 0, 3, 7, 9 and 13 days. In a second set of experiments, ECC-1 cells were incubated with estradiol for 5 days, then cultured with and without 1μM progesterone for 2, 4, 6 and 8 days. Maspin expression and progesterone receptor expression in the cell lysates were measured by western blot analysis using anti-maspin antibody and anti-progesterone receptor antibody. Results were normalized to actin expression.

Results: There was a time dependent decrease in maspin expression in ECC-1 cells cultured with estradiol. Maximal change in maspin expression occurred by 13 days compared to controls (52% reduction, see figure below). To investigate progesterone effects, cells were primed with estrogen for 5 days, and expression of progesterone receptors A + B was confirmed by western blot analysis. Progesterone alone did not appear to affect maspin expression over an 8 day span.

Conclusions: Our preliminary results suggest that estrogen may down-regulate maspin expression in ECC-1 cells. This confirms findings in breast tissue where estradiol negatively regulates maspin expression by binding to the hormone response element in the maspin gene promoter. During the proliferative phase of the menstrual cycle, when estradiol is the predominate hormone, we have previously demonstrated down-regulation of maspin expression. The precise role of maspin in the human endometrium still needs to be investigated.



241

Elevated Stress through SAPK/JNK and AMPK Regulate Nuclear Transcription Factors That Induce the First Endocine Factors during Development of the Placenta. Yufen Xie,[1] Simona Proteasa,[1] Jian Liu,[1] Elizabeth E Puscheck,[1] Wenjing Zhong,[2] Michael Diamond,[*1] Daniel A Rappolee.[1,2] *[1]Ob/Gyn, Wayne State University Medical School, Detroit, MI, USA; [2]Anatomy and Cell Physiology, Wayne State University Medical School, Detroit, MI, USA.*

Stress causes homeostatic responses such as slower cell cycle, cell cycle arrest, and apoptosis in preimplantation mouse embryos and in trophoblast stem (TS) cells. These responses are largely mediated by the stress-signaling enzymes, stress-activated protein kinase (SAPK) and AMP-activated protein kinase (AMPK). Both of these kinases are induced rapidly by stress and enter the nucleus. SAPK is necessary to induce and maintain nuclear HAND1 and AMPK is necessary to cause loss of nuclear ID2. Together these stress enzymes are necessary for the induction of placental lactogen 1 (PL1) through regulation of nuclear transcription factors. Therefore, stress, acting through stress enzymes, can take over development. But, is this just a quirk of a single ensemble of developmental problems? A global analysis of the kinetics of the stress response of TS cells suggests that the induction of PL1 protein is a small part of an entire developmental program activated by stress. The transcripion factors mediating early primary and secondary trophoblast giant cells are induced by 24hr of stress. These include hairy enhancer of split 1 (HES 1), retinoic activated 13 (STRA 13), and GATA2. But, transcription factors marking later-arising placental lineages of the inner placenta, spongiotrophoblasts (TPBPa, MASH2) and syncytiotrophoblasts (GCM1, TEF5) are not induced. Besides PL1, other

early endocrine factors are also induced. These include proliferin, PLP-M, and PLP-E, but not later endocrine factors such as PLII and PLP-A. Therefore, stress, acting through stress enzymes and then nuclear factors, can regulate TS cell differentiation into the first outer cells of the placenta producing the first endocrine hormones of the maternal recognition of pregnancy.

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242

Effects of IMD_{17-47} Infusion on Angiogenic Factors in Implantation Sites during Early Gestation in Rats. Madhu S Chauhan, Rebekah Elkins, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Intermedin (IMD)/Adrenomedullin (AM) 2 is a novel member of the calcitonin/ calcitonin gene-related peptide (CGRP) family. We have shown that IMD mRNA as well as protein are expressed in rat implantation site and that IMD antagonist (IMD_{17-47}) adversely affects rat implantation sites restricting its growth. Therefore, we hypothesize that IMD may be involved in angiogenesis during implantation and placental formation in early gestation in the rat.

OBJECTIVES: To analyze the effect of IMD_{17-47} on the expression of: 1) Hypoxia inducible factor-α [HIF1- α]; 2) angiogenic factors such as vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and Angiopoitin 1; and 3) sFLT-1 and Angiopoitin-2, in the rat implantation sites collected from IMD_{17-47} infused Day (D) 9 and control rats and assess the changes in their levels.

METHODS: Sprague Dawley rats were used in this study. Osmotic mini-pumps containing vehicle alone or IMD_{17-47} (200 μg/day) were inserted S.C. into pregnant rats on D3 of gestation and sacrificed on D9 (n = 5). Total RNA and protein was isolated from implantation sites using TRIzol reagent and processed for RT-PCR and Western blots. The results are expressed relative to 18S mRNA or β-Tubulin.

RESULTS: Our results demonstrate that: 1) IMD_{17-47} caused a significant decline in the expression of HIF1- α ($p<0.05$) in the implantation sites; 2) IMD_{17-47} significantly increased the expression of both VEGF and sFLT-1 mRNA ($p<0.05$); 3) IMD_{17-47} caused a significant decline in the levels of PLGF protein in implantation sites ($p<0.05$); and 4) levels of Angiopoitin-1 mRNA do not change in response to IMD_{17-47} but Angiopoitin-2 mRNA, an antagonist for Angiopoiten-1, is significantly increased in implantation sites of treated rats ($p<0.05$).

CONCLUSION: Hypoxia inducible factor-α (HIF1- α) a known regulator of VEGF production shows a significant decline in response to IMD_{17-47} treatment. Significant decline in HIF1- α caused by IMD_{17-47} infusion and an increase in the expression of the sFlt-1 and Angiopoitin- 2, an antagonist of the angiogenic factors VEGF and angiopoitin-1, respectively; indicates that endogenous IMD may be involved in angiogenesis of implantation site in early pregnancy.

243

Expression of a Mitochondrial Progesterone Receptor in Human Spermatozoa. Julierut Tantibhedhyangkul, Nikki Saner, Thomas M Price.* *Obstetrics and Gynecology, Duke University, Durham, NC, USA.*

Progesterone is an essential regulator of several female reproductive events. In contrast to the established roles of progesterone in the female, there are limited data on the role of progesterone in male. Reports have suggested a role for progesterone in various sperm functions, including capacitation, acrosome reaction, motility, egg penetration, and fertilization. Our laboratory has previously cloned and expressed a truncated progesterone receptor, named PR-M. PR-M, localized to the mitochondria by techniques including Western blot analysis after cellular fractionation of breast cancer cells, immunofluorescent localization of a GFP tagged recombinant PR-M and Western blot analysis of purified human heart mitochondrial proteins. Recent studies demonstrate progesterone induced changes in mitochondrial membrane potential. **OBJECTIVE:** The purpose of this study was to identify and localize PR-M in human spermatozoa. **METHODS:** Total RNA was isolated from washed human sperm with RNeasy. RT-PCR, using oligonucleotide primers specific to PR-M, was performed with subsequent PCR product sequencing. Immunofluorescent antibody staining was performed with confocal microscopy. Sperm were selected by density-gradient centrifugation, fixed and permeabilized. A primary anti-PR antibody (C19, Santa Cruz Biotechnology) directed to the hormone binding domain was used, with a normal rabbit IgG as a control. Mitochondria were stained with MitoTracker 580 (Invitrogen). Dual immunofluorescent imaging was performed at 488 and 580 nm. **RESULTS:** A PCR product extending from exon 1 to exon 2 of PR-M was identified. Indirect immunofluorescence of fixed sperm using C19 antibody showed the presence of a progesterone receptor in the midpiece. Staining of mitochondria showed co-localization with the PR staining. **CONCLUSIONS:** Identification of a specific transcript for PR-M and demonstration of PR localization in the mitochondria are consistent with the expression of this mitochondrial PR in human spermatozoa. We hypothesize that this receptor may provide a mechanism whereby progesterone within the female genital tract affects sperm energy production necessary for hypermotility and fertilization.

244

Expression of GLUT8, GLUT9a and GLUT9b in Mouse Testis and Sperm Is Altered in AKT1 and AKT2 Null Mice and May Be Associated with Normal Maturation and Fertilization. Sung Tae Kim, Kelle H Moley.* *Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA.*

Objective: The AKT signaling pathway plays critical role in glucose utilization of most cells. Glucose uptake into cells is facilitated by a family of glucose transporters (GLUTs). However, the specific role of GLUTs in the male reproductive system is not yet known.

Methods: To compare expression/localization of GLUTs in control, AKT1 null, and AKT2 null mouse testis and sperm, we analyzed the protein expression of GLUT8, GLUT9a, and GLUT9b in testis and epididymal sperm. In addition, we performed CASA and IVF with control oocytes in order to investigate motility and fertilization capacity of AKT1 null and AKT2 null sperm.

Results: GLUT8 was localized in innermost cells of seminiferous tubule and Leydig cells of all three testis groups. GLUT9a protein was not expressed in testis of AKT1 null and AKT2 null mice. GLUT9b expression was increased to about 30% in testis of AKT1 null and AKT2 null as compared to control testis and this was confirmed by western immunoblot analysis ($n=3$, $p<0.01$) In sperm, GLUT8 was localized in the acrosomal region, mid-piece, and principal piece of all three groups of sperm. GLUT9a localized to the mid-piece of normal sperm. Similar to the testis, GLUT9a protein was not expressed in AKT1 null and AKT2 null sperm. GLUT9b localized to the acrosomal region, mid-piece, and principal piece of sperm. Apoptosis of AKT1 null sperm was slightly increased (62/721, 8.6%) as compared to normal (11/227, 4.8%) ($n=2$). In AKT1 null males, sperm concentration and motility were significantly decreased; sperm concentration was 2×106/ml (normal was 82.7×106/ml) and motility was 9.5% (normal was 90%). AKT2 null sperm showed significantly abnormalities, but better than AKT1 null sperm. In vitro fertilization rates and developmental rates to blastocyst were also significantly decreased in AKT2 null male group respectively (52.2% and 13.5%) compared to control mice (88.5% and 64.5%) ($n=3$, $p<0.01$).

Conclusions: These results suggest that glucose uptake via GLUT8, GLUT9a, and GLUT9b may affect normal spermatogenesis and Leydig cell steroidogenesis in mouse testis. Due to differences in localization, we conclude that these transporters may play distinct roles in spermatogenesis in mouse sperm. In addition, the expression of AKT1 and AKT2 appears to play a critical role in GLUT9a protein expression, and as such may contribute to normal sperm function.

245

Mitochondrial DNA and Reproductive Aging. Rachel Forman, Andrea Jurisicova, Beth Acton, Robert Casper.* *Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada.*

Objective: To determine if point mutations in oocyte mitochondrial DNA (mtDNA) located within the NADH dehydrogenase subunit 2 (S2) and cytochrome b (CYTO) are associated with changes in mitochondrial membrane potential (DYm), maternal age, infertility diagnosis and cycle outcome.

Methods: 56 metaphase II unfertilized oocytes were obtained from patients undergoing ART treatment. Oocytes were divided into groups based on maternal age. The membrane potential was determined on live cells using DYm sensitive JC-1 (Depsipher) then stored at -80°C. The mutational survey in the oocytes was conducted by amplification of mtDNA with primers designed to span an area of known point mutations previously identified in individuals with heritable late-onset disorders such as essential hypertension and type II diabetes. Amplified products were sequenced from both ends and compared to a reference human mtDNA sequence.

Results: 56 oocytes underwent PCR amplification. In 64% of the oocytes one of the two studied amplicons was obtained and sequenced. 24 amplicons were generated and sequenced from the primer encompassing the S2 region. Six point polymorphisms/ mutations were identified within this area, the most frequent at 4769bp (91.7% of amplicons). One oocyte with a point mutation at

position 4917bp associated with Leber's Hereditary Optic Neuropathy (LHON) was also detected. 26 amplicons were generated from the primer encompassing the CYTO region. Eight point polymorphisms/mutations were identified. 96% of oocytes contained an identifiable polymorphism. The most frequent one, at position 15326bp, leads to a known amino acid change found in individuals with type II diabetes. Another point mutation was identified at 15257 bp and is associated with LHON.
The overall pregnancy rate was 20%. For patients achieving pregnancy mean age was 32.8 years and mean oocyte DYm ratio was 0.856. Patients who did not conceive had a mean age of 36.3 years and an elevated DYm ratio of 1.111. Mean number of polymorphisms/mutations was 1.5 in patients who conceived and 2.7 in those who did not. Mean number of polymorphisms/mutations was 2.0 in patients under 35 years and 2.4 in those aged 35 and over. In the younger group mean values were 1.4 for those who conceived and 2.7 for those who did not. Tubal obstruction was the diagnosis in 100% of patients who conceived and 62% of those who did not.
Conclusions: Oocytes of patients who conceived showed a trend toward lower DYm values and fewer mtDNA mutations.

246

Leptin Increases Aromatase Expression in Human Leiomyoma Cells Primarily Via the Jak-Stat Pathway and Aromatase Promoter I.4 Region.
Erica E Marsh, Zhihong Lin, Masashi Demura, Ping Yin, Eugene Xu, J Julie Kim,* Serdar Bulun.* *Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Chicago, IL, USA.*
Background: Leptin, the protein hormone secreted largely by adipocytes, increases with body mass index and has been shown to increase aromatase activity in various cell lines. Uterine leiomyomas are estrogen sensitive tumors and have increased prevalence in obese populations. We hypothesize that leptin may increase aromatase levels in leiomyomas thereby leading to increased local estrogen and accelerated growth.
Objective: To determine the signaling pathway and aromatase promoter region responsible for leptin-induced aromatase expression in leiomyomas.
Methods: Primary human leiomyoma cells were serum-starved and then treated with leptin at varying dosages (10-1000 ng/ml) for 48hrs. Real-time PCR was performed to identify the impact of leptin treatment on aromatase mRNA levels. Multiplex PCR was performed using primers recognizing promoter-specific aromatase mRNA species to determine which aromatase promoter was utilized in the leptin-induced increase. Western blots were performed on leptin treated protein lysates to evaluate the phosphorylation of the leptin receptor associated downstream effectors, Jak2 and Stat3. Leiomyoma cells were also co-treated with AG490, a Jak2 tyrosine kinase inhibitor, to determine if the effects of leptin would be reversed in the absence of activated Stat3.
Results: Total aromatase mRNA in leptin-treated leiomyoma cells increased in a dose-dependent fashion up to 15 fold ($p< 0.01$) versus control after 48hrs of treatment. Addition of AG490 blocked this increase. Western blots revealed that phosphorylated Jak2 and phosphorylated Stat3 levels were increased with leptin treatment in a time-dependent fashion. Multiplex PCR demonstrated that utilization of promoter I.4 increased strikingly by 35.47-fold in leptin treated cells versus vehicle treated cells ($p< 0.01$). There was a more modest increase in usage of promoter II by 11.53 times ($p<0.05$).
Conclusions: This study is the first to demonstrate that leptin increases aromatase expression in leiomyoma cells and this increase is mediated primarily via and the Jak2-Stat3 pathway and aromatase promoter I.4 that resides 73 kb upstream of the common coding region. Leptin receptor antagonists or Jak2/Stat3 inhibitors may serve as novel therapeutic agents by decreasing local estrogen production in leiomyoma.

247

The Comparison between the Human Blastocyst Secretome of Sequential Versus Coculture IVF Systems Points to IL-6 as a Predictor of Implantation.
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Objective Our group has developed a co-culture system with human endometrial epithelial cells (EEC) that improves blastocyst rate compared to sequential media. Our aim in this work is to describe the differential protein secretion and consumption pattern (secretome) of single human blastocysts cultured in sequential or coculture media.
M&M Using a single blastocyst transfer program, we compared sequential media (50µl of CCM™, Vitrolife) with co-culture media from day 5 human blastocysts with similar morphology (cultured 24h prior to transfer) using a protein array(Chemiarray®, Chemicon). Two experiments comparing a total of 20 blastocyst conditioned media (sequential n=10 vs coculture n=10) were analyzed in pools of 5 samples. As a control, we also pooled 5 samples containing only CCM with EEC. Data were analysed using Image J and GEPAS. We also used Fatiscan tool to find out functional terms (such as Gene Ontology and InterPro motifs). After this, we focus on IL-6 quantification in single blastocysts sequential media (n=54) by ELISA (Chemikine®, Chemicon) in order to validate data obtained from protein arrays.
Results. We obtained a differential protein profile in sequential media versus our co-culture model. Among 120 proteins studied, we find out that *cytokine binding* and *transmembrane receptor activity* proteins were increased in sequential media while *receptor binding proteins* were increased in the co-culture media. Interestingly, IL-6 (5.2 fold) and PlGF (3 fold) were the most increased proteins in the co-culture media versus sequential media. When we further analysed IL-6 concentrations by ELISA in sequential media from single blastocysts that implanted (270 pg/µl, n=20) versus those that did not implanted (1640 pg/µl, n=24) we obtained statistical differences between these two groups ($p<0.001$).
Conclusion: We have described the secretome of the human blastocyst cultured in sequential media versus EEC co-culture. We found that IL-6 is a differential predictor that discriminates the secretome of implanting versus non-implanting human blastocyst. These findings strongly suggest that the IL-6 concentration in conditioned media presumably secreted by the EEC monolayer may benefit not only embryonic development but also implantation ability.
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248

HOXA10 Represses BTEB1 Expression by Direct Promoter Binding.
Hongling Du, Hugh S Taylor.* *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objective: The Sp1/Kruppel-like family (KLF) member gene, BTEB1, directly interacts with PR to mediate progesterone-responsive gene expression in endometrium. We have previously shown that BTEB1 expression is lower in secretory than proliferative phase in endometrial glandular cells. In contrast, human endometrial HOXA10 expression is higher in the secretory phase. Here we show that BTEB1 was down-regulated by HOXA10 in endometrial Ishikawa cell line and demonstrate mediation by HOXA10 binding to single site in the BTEB1 promoter.
Methods: To determine whether HOXA10 directly regulated transcription of BTEB1, Ishikawa cells were transfected with artificial reporter constructs containing the BTEB1 promoter regions. Approximately 700bps of the BTEB1 5' upstream regulatory region was cloned. Nested constructs were amplified by PCR and cloned into pGL3 reporter vector. Additionally, mutant BTEB1 promoter sequences were generated and cloned. Ishikawa cells, grown to 50–60% confluence in 24-well plates, were transfected using Lipofectamine with 0.4 µg of pGL3-BTEB1 promoter vectors and cotransfected with 0.4 µg of empty pcDNA vector or pcDNA/HOXA10 expression vector. The pRL-TK vector was co-transfected as a control for transfection efficiency. After 48hs, the cells were harvested and Luciferase activity measured. Transfections were performed in duplicate and experiments were repeated three times. Electophoretic Mobility Shift Assay (EMSA) was used to confirm direct HOXA10 binding to the BTEB promoter.
Results: Transient transfection and luciferase reporter assay showed that luciferase activity was decreased by HOXA10 treatment through a single 149bp region. Compared to the control, the luciferase activity of the 700bps BTEB promoter reporter construct was decreased by 43% after HOXA10 cotransfection ($p<0.05$). This repression was localized to a 149bp BTEB proximal promoter region that demonstrated a 55% decrease in luciferase activity in response to HOXA10 treatment ($p<0.05$). No significant change in luciferase activity was obtained after treatment using the BTEB promoter region in which a putative HOXA10 binding site was mutated. In EMSA, HOXA10 was shown to directly bind this site.
Conclusion: BTEB1 was down-regulated by HOXA10 in endometrial cells. HOXA10 down-regulates BTEB1 expression by binding a single HOXA10 site in the BTEB1 promoter. HOXA10 likely regulates progesterone responsiveness through direct modulation of BTEB1 expression.

249

Toward Gene Therapy of Premature Ovarian Failure: Adenovirus Expressing Human FSH Receptor Corrects the Finnish C566T Mutation. Mohsen Ghadami,[1] Rebecca Chilvers,[1] Ayman Al-Hendy.*[1] *[1]Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA; [2]Ob/Gyn, University of Texas Medical Branch, TX, USA; [3]Ob/Gyn, University of Texas Medical Branch, Galveston, TX, USA.*

Introduction: Resistance Ovarian Syndrome (ROS), a common form of premature ovarian failure, characterized by primary amenorrhea in a normal karyotype female with an elevated serum levels of follicle stimulation hormone (FSH). A missense mutation, C566T, in follicle stimulating hormone receptor, FSHR, gene has been identified in numerous Finnish families and in several other countries.

Objective: To investigate if an adenovirus expressing a normal copy of human FSHR (Ad-FSHR) has the ability to: 1) transfect COS7 and JC410 cell lines, to render them responsive to FSH. 2) Restore FSH responsiveness in those cell lines that express the malfunctioning FSHR gene with C566T mutation.

Methods: Transfection efficiency of adenovirus was assessed by studying transfection of an ad vector carrying a marker gene, Ad-LacZ, followed by X-gal staining. Cells were transfeced with a plasmid containing mutated form (C566T) of FSHR gene. COS7 and JC410 cell lines were infected by Ad-FSHR. The presence of mutated and normal FSHR in transfected clones was tested by PCR. Functional activity of the Ad-FSHR was tested by: 1) Intracellular cAMP radioimmunoassay (RIA) of Ad-FSHR transfected cells, Ad-Lacz transfected, and untransfected cells. 2) Luciferase activity of JC410 expressing P450scc-luc and JC410 expressing StAR-luc cell lines transfected with Ad-FSHR or Ad-LacZ.

Results: Ad- LacZ transfection of COS7 and JC410 cell lines demonstrated a bright nuclear blue color. cAMP RIA indicated that untransfected cells, and a group of cells that expressing mutated FSHR had a minimal increase in cAMP levels after FSH stimulation, compare to 2 to 8-fold increase Ad-FSHR transfected cells. No significant changes observed in the cells transfected with Ad-LacZ. Luciferase measurement of JC410 cells expressing P450scc-luc and JC410 expressing StAR-luc transfected with Ad-FSHR showed a 2 to 3-fold higher luciferase transactivation compared to Ad-LacZ or untransfected cell.

Conclusion: A normal copy of hFSHR gene delivered via an adenovirus is able to transcomplement the Finish C566T mutated form and restore FSH responsiveness in porcine granulosa cells and COS7 cells. In vivo gene therapy with Ad-hFSHR in female FORKO mice is underway in our laboratory.

250

Genomic and Proteomic Analysis of Leiomyomas: Comparative Assessment in African Americans and Caucasians and with Other Fibrotic Disorders. Xiaoping Luo, Qun Pan, Nasser Chegini.* *OB/GYN, University of Florida, Gainesville, FL, USA.*

Clinical and epidemiological observations indicate that leiomyomas occur with higher frequency in African Americans (AA) as compared to other ethnic groups. For that reason and fibrotic characteristics leiomyomas have been often compared with keloids. To identify the molecular environments that differentiate leiomyomas in AA and their tissue-specific and common profiles with keloids/incisional scars and peritoneal scars we used microarray and 2D PAGE coupled with image and tandem mass spectrometric analysis. At the genomic level (Affymetrix U133A) analysis based on P≤0.01 and 2-fold cutoff change identified 1470 differentially expressed genes in leiomyomas regardless of ethnicity, of which 268 were either overexpressed (177) or under-expressed (91) in leiomyomas of AA as compared to Caucasians. The analysis also identified 424 and 393 genes as over- or under-expressed in leiomyomas of AA and Caucasians as compared to keloids/incesional scars, respectively, with only 85 differentially expressed genes with peritoneal adhesions. The gene expression profiles in these tissues were comparatively analyzed with their corresponding normal tissues, myometrium, skin and peritoneum, and as expected the analysis revealed distinct differences. The expression of 12 of these genes; RUNX3, E2F1, EGR3, FBLN5, COL18, CST6, GAS7, ECM2, ADAM17, THBS1, ESM1 and TBPIP was validated in these tissues using microfluidic arrays and realtime PCR. At protein level 332 protein spots were identified of which average density/volume of 31 spots varied by ≥1.5 fold in leiomyomas regardless of ethnicity. In leiomyomas the density/volume of 34 proteins of these spots differ (26 overexpressed and 8 underexpressed) in AA as compared to Caucasians. MS/MS analysis of 15 spots resulted in identification of several proteins whose transcripts were also identified by gene profiling, of which 14-3-3ß and mimecan confirmed by western blotting and immunohistochemistry. These results provided the first large scale molecular assessment of leiomyomas suggesting that a) the level of expression rather than ethnic-specific genes accounts for the difference between leiomyomas in AA and other ethnic groups and b) combination of common and tissue-specific genes with altered level of expression compared to their normal tissues, accounts for the shared fibrotic characteristics between leiomyomas, keloids and other fibrotic tissues. (Supported by grant HD37432).

251

Maternal Ghrelin Deficiency Compromises Reproduction in Female Offspring. Sarah B Lieber, Maria Shanabrough, Tamas L Horvath, Hugh S Taylor.* *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: First recognized for its roles in growth and appetite, ghrelin has since been implicated in reproduction. Reduction in murine litter size and restriction of embryonic growth have been demonstrated in response to ghrelin administration. Here we investigate the consequences of maternal ghrelin deficiency on fertility of female offspring.

Methods and Results: Litter sizes were analyzed for mating events between wild-type male and female ghrelin +/+ mice parented by ghrelin +/- B6D2F1 hybrid mice. Comparison of ghrelin +/+ exposed females with wild-type controls revealed significantly reduced litter sizes among ghrelin +/+ females exposed in utero to maternal ghrelin deficiency (n = 5, 4.7 ± 3.3 pups for in utero deficient ghrelin +/+ females; n = 4, 8.7 ± 2.3 pups for wild-type females, p = 0.010 by t-test). Oocyte and corpus luteum number was similar between groups, suggesting a uterine defect. The ghrelin receptor (GHSR) was expressed in uterine endometrium as determined by RT-PCR. Immunohistochemical staining of uterine sections obtained during estrus demonstrated increased expression of proliferating cell nuclear antigen and more uniform expression of common leukocyte antigen in stroma and epithelia of ghrelin +/+ females exposed to in utero ghrelin deficiency relative to wild-type controls. As HOX genes regulate uterine development, we assessed the effect of ghrelin on endometrial cell HOXA10 expression. Treatment of Ishikawa cells with 1 nM, 10 nM, and 100 nM ghrelin for 24 h produced 5.82-fold, 6.15-fold, and 5.62-fold elevations, respectively, in expression of HOXA10, as determined by qRT-PCR.

Conclusion: We demonstrate that maternal ghrelin deficiency compromises reproduction in female offspring. Ghrelin affects the expression of genes that regulate uterine development. In utero ghrelin deficiency may lead to anomalous differentiation and subsequent uterine dysfunction. While ghrelin is known to affect the HPO axis in the adult, in utero it likely also contributes to regulation of reproductive tract development. These findings have significant implications for maternal nutrition, energy balance, and maintenance of appropriate ghrelin expression in pregnancy; deficient maternal ghrelin related to obesity may progam the development of the female reproductive tract.

252

Essential Nutrient Supplementation Preventing Adult Metabolic Disease in a Transgenerational Model of IUGR Is Accompanied by Differential Alterations in Gene Expression. Kjersti M Aagaard-Tillery,[1] Robert McKnight,[2] William Holland,[3] Xingrau Ke,[2] Xi Fu,[2] Michael Varner,*[1] Robert Lane.[2] *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA; [2]Pediatrics, University of Utah, Salt Lake City, UT, USA; [3]Biochemistry, University of Utah, Salt Lake City, UT, USA.*

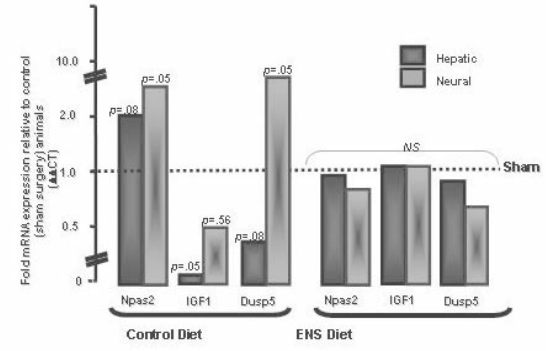
OBJECTIVE: We have previously shown that development of the adult metabolic phenotype in response to a constrained *in utero* environment is associated with altered fetal one-carbon metabolism. This manifests as persistent changes in gene expression accompanied by epigenetic modifications. Given that supplementation with essential nutrients (ENS) prevents adult obesity and hepatic insulin resistance in our heritable transgenerational model of IUGR, we sought to characterize the effect of ENS on expression of epigenetically reprogrammed genes.

STUDY DESIGN: Sprague-Dawley P1 dams underwent bilateral uterine artery ligation or sham surgery on e19, and resultant F1 litters yielded IUGR or control lineages, respectively. On d21, weaned F1 were allocated to ENS (Teklad8640+folic acid/choline/B12/betaine/L-methionine/L-arginine/zinc) or control diet (Teklad8640). F1 pairs were mated by d80, and resultant F2 (n 512) were weaned to their parental diet. Expression of fetal genes found to be reprogrammed under an altered *in utero* environment were examined: *IGF1,* a circadian regulator *Npas2,* and the Erk dual-specificity phosphatase *Dusp5*.

RESULTS: In our model of transgenerational growth restriction, significant phenotypic differences in F2 offspring of IUGR lineages (maternal and/or paternal) when compared with shams are accompanied by altered postnatal

(d28) expression of *Npas2*, *IGF1* (exon4/6), and *DUSP5 (Fig. 1)*. Abrogation of such altered expression accompanies ENS-mediated prevention of the adult metabolic phenotype *(Fig. 1)*.

CONCLUSION: Diet supplemented with essential nutrients, yet unaltered in its caloric content, prevents adult obesity and insulin resistance in a heritable transgenerational model of IUGR. This is accompanied by significant alterations in the expression of reprogrammed metabolic genes.



253

Maternal Food Restriction during Pregnancy Affects Hepatic IGF-1 mRNA and Chromatin Structure in the Adult IUGR Offspring. Darran N Tosh,[1,2] Lane Robert,[3] Christopher W Callaway,[3] Robert A McKnight,[3] Isabela McMillen,[4] Michael G Ross,*[1] Mina Desai.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Adelaide, Australia; [3]Dept. of Pediatrics, University of Utah, Salt Lake City, UT, USA; [4]Sansom Research Inst, University of South Australia, Adelaide, Australia.*

OBJECTIVE: Maternal food restriction (MFR) during pregnancy causes IUGR offspring that when fed ad libitum show catch-up growth by 3 weeks and develop obesity and metabolic syndrome as adults. Postnatal insulin resistance is a characteristic of the IUGR offspring and hepatic IGF-1 may play a role in the development of these morbidities. IGF-1 is epigenetically regulated involving two different promoters, alternative exon splicing of exons 2 and 5, and multiple transcription termination sites. We determined IGF-1 hepatic mRNA levels and epigenetic characteristics in the MFR offspring.

STUDY DESIGN: Control Sprague Dawley dams (n=5) received ad libitum food, whereas study dams (MFR, n=5) were 50% food-restricted from pregnancy day 10 to 21 resulting in IUGR newborns. At birth litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned to ad libitum feed. Male plasma and livers were collected at 3 weeks and 36 weeks. Plasma levels of IGF-1 (RIA) and IGF-1 mRNA variant levels (real time RT-PCR) were quantified. Chromatin immunoprecipitation (ChIP) was subsequently performed using the antibody for H3K4 dimethyl, and associated levels of each IGF-1 species were measured by PCR.

RESULT: Plasma IGF-1 was not altered in MFR at 3 weeks of age but at 36 weeks was increased (% of control: 222%, $p<0.01$). Consistent with plasma levels, IGF-1 mRNA was not altered at 3 weeks, and at 36 weeks there was an increase in IGF-1A, IGF-1B, IGF-1 Exon 1 and IGF-1 Exon 2 (143.5%, 183.3%, 146.5%, 149.4%, $p<0.001$). Analysis of DNA from ChIP with dimethyl H3K4 found no effect at 3 weeks and a decrease at 36 weeks of the IGF-1 P1 promoter (62.6%, $p<0.01$) and exon 5 (49.4%, $p<0.05$).

CONCLUSION: Adult MFR IUGR males have increased postnatal hepatic IGF-1 mRNA levels and plasma IGF-1 with decreased H3K4 dimethylation at IGF-1. We speculate the IUGR alters IGF-1 mRNA in the MFR adult offspring by affecting IGF-1 histone and chromatin structure, possibly through histone modifications other than H3K4 dimethylation.

254

***In Vivo* Effects of Antioxidants on Vascular Reactivity in the Late Gestation Fetus.** AS Thakor, Dino A Giussani.* *Physiology, Cambridge, United Kingdom.*

Introduction: Prenatal vitamin supplements are prescribed worldwide by doctors to help expectant mothers meet the extra nutritional requirements during pregnancy and lactation. Formulations usually include folic acid and antioxidants. While the beneficial effects of folic acid in guarding against birth defects is established, the effects on the fetus of exposure to antioxidants is much more uncertain. This study investigated the effects of fetal exposure to two different antioxidants on the cardiovascular physiology of the late gestation fetus during basal and stimulated conditions.

Methods: Under anesthesia, 25 fetal sheep were surgically prepared with vascular catheters and a Transonic flow probe around one femoral artery at 0.8 of gestation. Five days later, *in vivo* pressor and vasopressor responses were elicited to increasing i.a. bolus doses of phenyleprine (5-50 microg) before and during fetal i.v. treatment with either melatonin (n=7; 0.5±0.1 microg/kg/min) or vitamin C (n=6; 8.9±0.4 mg/kg/min). The remaining 12 fetuses were subjected to 30 min of acute hypoxemic stress (reduced maternal Fi,O2) during saline or fetal i.v. treatment with melatonin (n=6) or vitamin C (n=6).

Results: Fetal exposure to either antioxidant markedly diminished the pressor and vasopressor responses to alpha adrenergic stimualtion (Fig. 1) and to hypoxemic stress (Fig. 2).

Conclusion: Exposure of the late gestation fetus to antioxidants has pronounced effects on *in vivo* vascular reactivity during basal and stimulated conditions. Prescription of prenatal antioxidants to expectant mothers should take these findings into consideration.

Supported by The Lister Institute for Preventive Medicine and The Journal of Experimental Pathology.

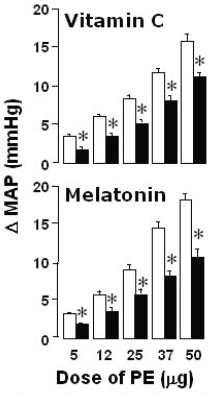


Fig.1. Mean+SE for the increment in fetal arterial pressure (MAP) to PE during saline (□) or antioxidant (■) treatment. *P<0.05, ANOVA.

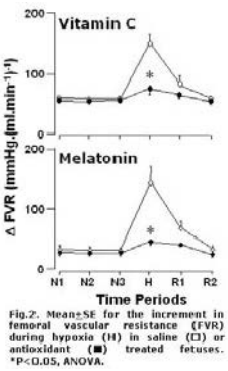


Fig.2. Mean±SE for the increment in femoral vascular resistance (FVR) during hypoxia (H) in saline (□) or antioxidant (■) treated fetuses. *P<0.05, ANOVA.

255

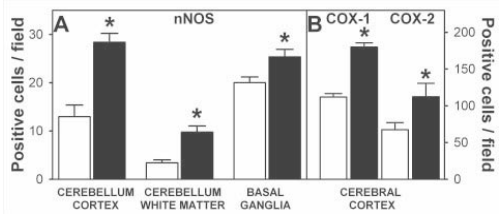
Chronic Mild Hypoxemia Alters the Expression of Nitric Oxide- and Prostaglandins-Generating Enzymes in Near-Term Fetal Sheep Brain. Victor M Pulgar, Jing Wang, Jie Zhang, Angela G Massmann, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston Salem, NC, USA.*

OBJECTIVE: Nitric oxide and prostaglandins are important mediators of the fetal response to hypoxemia and asphyxia. We have previously reported that 5-days of mild chronic hypoxemia increases neuronal death 72 h after four 5-min complete umbilical cord occlusion (UCO) in near-term fetal sheep. The aim of the present study was to ascertain if there were differences in the expression of nitric oxide and prostaglandins-generating enzymes in three different areas of the fetal brain vulnerable to hypoxic damage.

METHODS: At 125±1 days, instrumented fetuses were submitted to a 5 days period of hypoxemia (75% of baseline arterial blood PO_2). After 5 days animals were submitted to four 5-min complete UCO with 30 min recovery in between. 72 h after UCO, brains were isolated and cryopreserved. 30μm–coronal sections were used (hypoxic n=5, control n=5) to evaluate the expression of nNOS, COX-1 and COX-2 in cerebral cortex, basal ganglia and cerebellum. Immunohistochemistry with ABC staining kit and peroxidase was used. Positive cells per field (200x) were recorded for each antibody used. Averages were compared by unpaired *t*-test with statistically significant difference considered if $P<0.05$.

RESULTS: NOS and COX-2 positive cells displayed a typical neuronal morphology. A greater number of positive cells for nNOS was observed in basal ganglia and in white matter and cortical areas of the cerebellum ($P<0.05$) of hypoxic fetuses (Fig 1A) whereas no differences were found in cerebral cortex. COX-1 and COX-2 (Fig 1B) showed increased expression in cerebral cortex of hypoxic animals ($P<0.05$) with no differences in basal ganglia and cerebellum.

CONCLUSIONS: Chronic hypoxemia modifies in a region-specific manner the expression of nNOS and COX enzymes. Up-regulation of nNOS in the striatum may be related to the increased neuronal death we observed in this region with this protocol of asphyxia. Increased expression of COX enzymes could be related to their role in asphyxia-induced inflammatory processes. NIH HD37885.



256

Expression of Hypoxia Inducible Factors (HIF) 1α, 1β and IGF2 in the Adrenal, Liver and Lung of the Chronically Hypoxic Fetal Sheep. Sheridan Gentili, Janna L Morrison, I Caroline McMillen. (SPON: David M Olson). *Early Origins of Adult Health Research Group, Sansom Institute, University of South Australia, Adelaide, South Australia, Australia.*

Objective: Exposure to chronic fetal hypoxia results in the sparing of growth of key organs such as the brain and adrenal and the relative slowing of growth of other tissues including the fetal liver. Whilst it is established that IGF2 is a major fetal growth factor, it is not known if chronic hypoxia acts via the hypoxia inducible factors (HIF) to differentially regulate IGF2 expression in the fetal adrenal (growth spared) compared to the fetal liver (growth slowed). HIF transcription factors are heterodimers consisting of a HIFα (1α, 2α and 3α) subunit bound to a HIF1β subunit, which act via the hypoxia response element of target genes, including IGF2.

Hypothesis: We hypothesise that HIF1α and HIF1β mRNA expression will be differentially regulated in tissues in which growth is spared (adrenal), slowed (liver) or unchanged (lung) in the chronically hypoxic fetus and that these changes will relate directly to changes in IGF2 mRNA expression.

Methods: Carunclectomy was performed in 12 non-pregnant ewes to induce placental and hence fetal growth restriction (PR fetuses). Vascular catheters were inserted in 12 PR and 12 control (C) fetuses at 103-117d and arterial blood samples were collected for blood gas analysis at frequent intervals until post mortem at 140-145d. The expression of HIF1α, HIF1β and IGF2 mRNA was determined using real-time RT-PCR.

Results: Fetal weight (C, 4.5±0.2kg; PR, 2.7±0.3kg) and mean gestational PO_2 (C, 20.7±0.9; PR, 13.7±0.5mmHg) were significantly lower in PR fetuses ($P<0.05$). Relative lung weight was maintained, whilst relative adrenal weight was higher and relative liver weight was lower in PR fetuses ($P<0.05$). The expression of HIF1α in the adrenal, lung and liver was not different between C and PR groups, whereas the expression of HIF1β was higher in the adrenal of the PR group compared to the C group ($P<0.05$). There was a positive correlation between HIF1α and HIF1β in the adrenal ($R^2=0.34$, $P<0.05$), lung ($R^2=0.35$, $P<0.01$) and liver ($R^2=0.53$, $P<0.05$). HIF1α expression was positively correlated with IGF2 expression in the adrenal ($R^2=0.62$, $P<0.05$).

Conclusions: The relationship between HIF1α and IGF2 in the fetal adrenal, an organ in which growth is spared in chronic hypoxia, suggests that HIF1α may play a role in mediating the effects of hypoxia in the regulation of IGF2 expression and adrenal growth.

257

Effect of Long Term Hypoxia on Glucocorticoid Receptor Protein Expression and Distribution in Ovine Fetal Adrenals. Brandon Root,[1,3] Jenna Abrassart,[1] Tshepo Monau,[3] Dean A Myers,[*2] Charles A Ducsay.[*3] *[1]Dept. Biol, Univ. of Redlands, Redlands, CA; [2]Dept. Ob/Gyn, Univ. of Oklahoma Health Sci. Ctr, Oklahoma City, OK; [3]Ctr. for Perinatal Biol, Loma Linda Univ, Loma Linda, CA.*

Background: Glucocorticoids play a key role in regulation of catecholamine synthesis, and may also play a role in adrenal responsiveness to ACTH. These effects are mediated through the glucocorticoid receptor (GR). Previous studies from our laboratory showed that long term hypoxia (LTH) significantly alters catecholamine and cortisol responses in the ovine fetus. This study was designed to test the hypothesis that LTH alters adrenal GR protein expression and/or distribution.

Methods: Pregnant ewes were maintained at high altitude (3,820m) from 30 to 138~140 days' gestation. Animals were then transported from high altitude to Loma Linda University when a maternal tracheal catheter was used to infuse nitrogen and maintain a reduced PO_2 at a level comparable to high altitude. Age matched normoxic fetuses were used as controls. At 139-141 days' gestation, fetal adrenals were collected. The medulla and cortex were separated and individually processed for GR Western analysis. Immunohistochemical localization of GR was performed in additional adrenals.

Results: There were no differences in GR in the medulla. In the cortex, 2 forms were observed; one consistent in molecular weight with the GR (95kDa) and a 45kDa form (values=relative optical densities).

	95kDa	45kDa	95kDa:45kDa
Control (n=6)	0.62±0.09	0.55±0.12	1.71±0.44
LTH (n=6)	0.56±0.13	0.65±0.11	0.96±0.16

Localization of GR in the medulla showed even distribution in both groups. In the cortex from control fetuses, heavy GR staining was observed in the zona glomerulosa and innermost zone with only light staining in the zona fasciculata. In the LTH adrenals, heavy, even staining was observed throughout the entire cortex.

Conclusions: The 45 kDa form of the GR has previously been reported and proposed to be a proteolytic fragment of the GR or arises via an alternative translation initiation codon. Preabsorption with synthetic GR peptide eliminated this band, confirming its specificity to the GR. The trend towards a decreased 95:45 kDa ratio in the LTH group suggests increased GR turnover in the adrenal cortex of these fetuses. Together with the differences in adrenal cortical localization, it appears that LTH alters GR function in the fetal adrenal cortex. (Supported by HD31226).

258

Deletion of the TNF Cluster Abolishes Lipopolysaccharide Sensitization to Hypoxia-Ischemia in the Brains of Neonatal Mice. Giles Kendall,[1] Gena Raivich,[1] Mariya Hristova,[1] K Pfeffer,[2] S Nedospasov,[3] Donald Peebles.[*1] *[1]Centre for Perinatal Brain Research, University College London, United Kingdom; [2]Institute of Medical Microbiology, University of Dusseldorf, Germany; [3]Engelhardt Institute of Molecular Biology, Moscow, Russian Federation.*

Experimental studies demonstrate a sensitizing effect of endotoxin (LPS) on the developing brain when given prior to hypoxia-ischemia (HI); the most severe brain injury is observed with a time interval of 12 hours between LPS and HI. To test the hypothesis that upregulation of pro-inflammatory cytokines contributes to this synergistic interaction we examined the role of Tumor Necrosis Factor (TNF) cluster, consisting of TNFa, lymphotoxin a (LTa) and lymphotoxin b (LTb) using transgenic mice.

Animals heterozygous for a deletion of the entire TNF cluster were bred and their offspring were used at postnatal day 7. HI was achieved by left carotid occlusion under isoflurane, followed by exposure to 8% oxygen for 30 minutes. LPS from *E.coli* (serotype 055:B5) or vehicle (normal saline) was administered intraperitoneally 12 hours prior to HI. At 48 hours the brains were perfused, extracted and post-fixed. Animals were genotyped using PCR and the brains of knockout and wildttype were evaluated. Infarct volumes are expressed as a percentage of the contralateral (uninjured) hemisphere.

Pretreatment with LPS 12 hours prior to HI increased infarct size in wildtype mice from 3% to 22%. No increase in infarct size was seen in the animals with deletion of the TNF cluster (1% saline pre-treated, 4% LPS pre-treated). When

the regions were analysed separately LPS sensitisation was demonstrated in the wildtype animals in all forebrain regions studied. Knockout of the TNF cluster abolished LPS sensitisation in all forebrain regions (see Fig 1).
These data show that the TNF cluster plays an important role in the neurotoxic interaction between LPS and HI. This partly explains the known correlation between pro-inflammatory cytokines (including IL-1b, IL-6 and TNFa) and brain injury and recent data showing an association between LTa polymorphisms and cerebral palsy (Nelson et al., Ped Res 57(4):494, 2006).

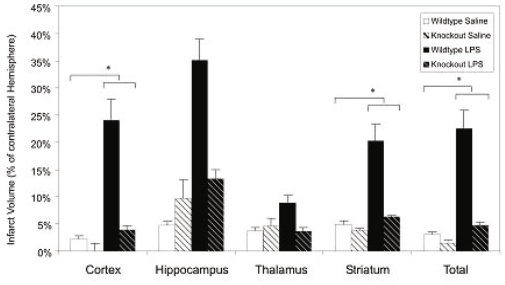


Fig 1: Effects of LPS pretreatment 12 hours prior to hypoxia ischemia in wildtype and TNFcluster knockout mice
* P<0.05

259

The Effect of Mechanical Stretch and Interleukin-1beta on Pre-B Cell Colony-Enhancing Factor Expression in Human Myometrium. Suren R Sooranna,[1] Peta Grigsby,[2] Zhiqing Liang,[1] Leslie Myatt,[*2] Phillip R Bennett,[*1] Mark R Johnson.[*1] *[1]Obstetrics & Gynaecology, Imperial College Parturition Research Group, London, United Kingdom; [2]Obstetrics & Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*
Introduction: Pre-B cell colony-enhancing factor (PBEF), also known as visfatin, is a 55 kDa protein that is present in myometrium. PBEF is a cytokine linked to the initiation of labour and up-regulation of the PBEF gene by the distension of amnion cells has been demonstrated. Increased PBEF expression in the fetal membranes is seen in labour, and inflammatory stimuli such as LPS, TNFα, Il-1β, and Il-6 all up-regulate PBEF gene expression. Our aim was to determine the effects of stretch and Il-1β on PBEF gene expression in the myometrium.
Methods: Primary human uterine myocytes were cultured in 6 well plates. When 90% confluent cells were serum starved overnight and incubated with 1ng/mL Il-1β for 6 hours. Uterine myocytes were also cultured in 6-well flexible-bottom plates pre-coated with collagen type I. When cells were 90% confluent, they were subjected to static stretch of 11% for 1h using a flexercell strain unit. Inhibition of ERK, JNK and p38 was achieved by incubation of cells with 10μM U0126 for 2h, 20μM SP600125 for 1h or 10μM SB203580 for 30 min respectively prior to stimulation. At the end of incubations RNA was extracted and converted to cDNA. Paired upper and lower segment myometrial tissue was collected at caesarean section either before or after the onset of term or pre-term labour and frozen for extraction of RNA (n=9 for PTNL;PTL; n=8 for TNL; n=10 for L). Copy numbers of PBEF, GAPDH and beta-actin were measured by quantitative PCR.
Results: 1h of stretch increases PBEF by 114% and this is unaffected by treatment with MAPK inhibitors. 6 h incubation of myocytes with 1ng/mL Il-1β caused a marked increase in PBEF by 385% (n=6; p<0.028) and this is unaffected by JNK inhibitor but partially decreased by either the ERK or p38 inhibitors. PBEF expression was similar in the upper and lower segment myometrium in preterm patients. In term lower segment myometrium the PBEF: beta-actin mRNA ratio was significantly greater in term labour (160.65±50.07) versus term non labour (6.75±2.23) samples (mean ± SEM; p<0.026).
Conclusions: These data show PBEF is increased by Il-1β in human myometrium in part by first activating ERK and/or p38 MAPK. PBEF expression increases markedly in lower segment myometrium in term labour.

260

Diminished Elafin Levels in Bacterial Vaginosis in Pregnancy and Production by Cells Derived from the Vagina and Cervix. Sarah JE Stock,[1] Rodney W Kelly,[2] Simon C Riley,[1] Andrew A Calder.[1] (SPON: Hilary OD Critchley). *[1]University Department of Obstetrics and Gynaecology, Queen's Medical Research Institute, Edinburgh, Scotland, United Kingdom; [2]Medical Research Council Human Reproductive Sciences Unit, Queen's Medical Research Institute, Edinburgh, Scotland, United Kingdom.*
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Aim: Elafin is a protease inhibitor with antibacterial and antiviral properties, which also modulates the adaptive immune response. Our aim was to examine vaginal and cervical production of elafin, and see if amounts were altered in pregnant women with bacterial vaginosis.
Methods: Cervicovaginal secretions were collected from women in the second trimester of pregnancy (n=113). Bacterial vaginosis status was assessed by Gram stain using Nugent's criteria. Enzyme liked immunosorbance assay (ELISA) was used to measure elafin and cytokines. Immortalized cell lines derived from the vagina, ectocervix and endocervix of one patient formed an *in vitro* model. Cells were treated with the bacterial wall products lipopolysaccharide (LPS) or lipoteichoic acid (LTA), or the inflammatory cytokine interleukin-1 beta (IL-1ß). Elafin and cytokine expression were determined by Taqman quantitative PCR and ELISA. Statistical significance was determined using the Kruskall-Wallis test and $p<0.05$ was regarded as significant.
Results: 58.5% (66/113) of women had normal vaginal bacterial flora, whilst 23.9% (27/113) had bacterial vaginosis, and 17.7% (20/113) had an intermediate flora. There were no significant differences between the groups in terms of age, racial mix or parity. Significantly lower levels of elafin were found in cervicovaginal secretions of women with bacterial vaginosis ($p<0.05$). Higher levels of IL-1ß and IL-1 receptor antagonist (IL-1RA) were also associated with bacterial vaginosis ($p<0.05$). Production of elafin was greater in vaginal cells than in ectocervical or endocervical cells ($p<0.05$). LPS upregulated both elafin mRNA and protein expression in endocervical cells ($p<0.05$) but did not effect expression in vaginal or ectocervical cells.
Conclusion: Elafin is produced by the vagina and cervix and may have an important role in defence against infection. The decreased amounts of elafin found in bacterial vaginosis may predispose to the condition, or contribute to its sequelae which include preterm rupture of membranes and preterm labour, and increased transmission of HIV.

261

Ethnic Disparity and Molar Imbalance in Amniotic Fluid Concentrations of TNF-α and Soluble TNF Receptors in Preterm Birth. Ramkumar Menon,[1,2] Poul Thorsen,[2] Ida Vogel,[2] Scott M Williams,[3] Stephen J Fortunato.[1] (SPON: Kelle H Moley). *[1]The Perinatal Research Center, Nashville, TN, USA; [2]North Atlantic Neuro Epidemiologic Alliance, Aarhus University, Aarhus, Denmark; [3]Department of Medicine, Vanderbilt University, Nashville, TN, USA.*
OBJECTIVE: Preterm birth rate (PTB) is higher in African Americans (AA) than Caucasian (C). Despite the fact that the overall rate of disparity is decreasing, the difference is still significant and the overall rate of PTB is increasing (~10% in the mid 1980s to ~12% now). It has been hypothesized that a differential inflammatory response may explain this disparity. This study examines the inflammatory cytokine, tumor necrosis factor–alpha (TNF-α), and soluble TNF receptor concentrations (sTNFR1 and sTNFR2) in the amniotic fluid (AF) of AA and EA women at delivery in a case-control study
DESIGN, SETTING AND PATIENTS: AF samples were collected (333) during active labor (158 spontaneous PTB, 52 AA and 106 C, gestational age $\leq 36^{0/7}$ weeks) and 175 controls ($>37^{0/7}$ weeks, 87 AA and 88 C). AF TNF-α and sTNFR1 and sTNFR2 concentrations were measured by immunoassay. Differences between cases and controls were analyzed using non parametric Wilcoxon signed Ranked test. The molar ratios of TNF-α to its receptors were compared between cases and controls within each ethnic group.
RESULTS: TNF-α concentration was higher in cases vs. controls when C and AA were analyzed together ($p= 0.0001$). There were no differences in total sTNFR (R1+R2) or sTNFR1 and sTNFR2 concentrations between cases and controls. However, AA cases had higher TNF-α concentration than AA controls ($p=0.0001$), but C cases did not differ from C controls ($p=0.2$). TNF-α was also higher in AA cases with microbial invasion of the amniotic cavity (MIAC) compared to AA cases with no MIAC ($p=0.0009$). This was not observed in C ($p=0.8$). There was no difference in total sTNFR concentration in AA ($p = 0.08$) or C($p=0.5$) cases vs. controls. The molar ratio of TNF-α/sTNFR (R1+R2) was 20 fold higher in AA cases than in AA controls in favor of TNF-α. This was not observed in C.

CONCLUSIONS: The results suggest substantial ethnic differences in one important hypothesized pathway that can affect pregnancy outcome. The TNF-α/sTNFR profile in pregnancy differs between ethnic groups, suggesting a difference in bioavailability of TNF-α. The larger molar ratio of TNF-α/sTNFR in AA cases may be indicative of a TNF-α mediated pathological process of PTB in AA as compared to C.

262

Polymorphisms in MMP-7 and MMP-8 Promoters Are Associated with Increased Risk of PPROM in a Hispanic Population. Pedro Ferrand,[*1,2] Claudia Arriagada,[2] Alfredo Ovalle,[1] Carolina Urbina,[1] Fernando Uribe,[1] Soledad Henriquez.[2] *[1]Obstetricia/Ginecologia Campus Central, Universidad de Chile, Santiago, Chile; [2]Instituto de Investigaciones Materno-Infantil, Universidad de Chile, Chile.*

Preterm Premature rupture of the membranes (PPROM) is a major cause of preterm birth and perinatal morbidity/mortality. Higher levels of Matrix Metalloproteinases (MMPs), as MMP-7 and MMP-8, have been described in amniotic fluid of patients with PPROM. Recent investigations of tissue remodeling of membranes have revealed that genetic variants that is associated with a changed expression of those proteases. Increased levels of MMP-7 and MMP-8 leading to rupture of membranes and labor, therefore, cause a preterm delivery. Objectives The goals of this study was to determine if functional polymorphisms in the MMP-7 promoter and MMP-8 promoter, with higher expression of both proteases, are associated with PPROM (-181 A/G and -153 C/T in MMP-7, and -799 C/T and +17 C/G in MMP-8). Methods: A case control study was conducted on neonates with cases defined as pregnancies complicated by fetal membrane rupture prior to 37 weeks of gestation. Controls were singleton pregnancies delivered at term with no prior history of preterm birth. Results: We found that the fetal carriage of the -799 T allele in MMP-8 was significantly associated with PPROM (OR 2.42 95%CI 1.01-5.79), that association was not find it in the +17G allele, However when we did the haplotype analysis we found a higher risk of PPROM when both polymorphisms were present (-799T/ +17G, OR 4,58 CI 95% 1.73-12.1). In the MMP-7 promoter the 2 polymorphisms were not associated with PPROM. When we include the haplotype that contains both promoter variants -799T/+17G for MMP-8 and -181G/-153T for MMP-7 we found a higher risk of PPROM (OR 8,42 CI 95% 1,2-60) Conclusions: We conclude that 1) fetuses who carry the -799 T allele in MMP-8 promoter have a greater risk of PPROM; 2) fetuses who carry the Haplotype -799 T/+17 G for MMP-8 have a greater risk of PPROM; 3) the MMP-7 alleles are not related to PPROM, and 4) Fetuses that carry the haplotype with the variants in MMP-7 and MMP-8 promoter have risk of PPROM.

263

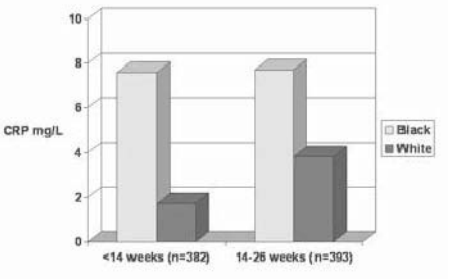
Black Women Have Higher Levels of Systemic Inflammation in Pregnancy as Measured by Serum C-Reactive Protein. Amy H Picklesimer,[1] Kevin Moss,[2] Steven Offenbacher,[2] James Beck,[2] Kim Boggess.[*1] *[1]Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [2]Dental Ecology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

OBJECTIVE: C-reactive protein (CRP) is an acute-phase reactant produced in response to stress, tissue injury and other inflammatory stimuli. In non-pregnant patients, subclinical elevation (>3.0 mg/L) is a marker for endothelial damage, atherogenesis and cardiovascular disease. During pregnancy, inflammation plays an important role in pregnancy-specific conditions such as pre-eclampsia and preterm labor. We sought to characterize CRP values in a diverse population of healthy pregnant women using a highly sensitive assay.

STUDY DESIGN: Cross-sectional study of 775 pregnant women enrolled prospectively in a study of oral health in pregnancy. CRP studies were performed on serum specimens drawn before 26 weeks gestation using commercially available highly sensitive ELISA kits. For analysis, the cohort was stratified by trimester of pregnancy and by maternal race.

RESULTS: Median CRP value for all patients was 4.8 mg/L (inter-quartile range 0.63 – 15.7). Median CRP value was significantly higher in the second compared to the first trimester (6.24 vs 3.94 mg/L, p=.0057). Blacks had higher median CRP values than whites (7.68 mg/L vs 2.59 mg/L, p<.0001). CRP values increased from the first to the second trimester for white (1.72 vs. 3.83 mg/L, p=.007) but not black women (7.61 vs. 7.72, p=1.00). Blacks had significantly higher median CRP values for both the first and second trimester when compared with whites (p< .0001). This relationship persisted even after controlling for known confounders such as maternal weight.

CONCLUSION: Pregnancy is an inflammatory state as measured by CRP. Elevations manifest early in gestation, and persist throughout pregnancy. Blacks have higher levels of serum CRP when compared to whites, which is measurable from even the earliest gestational ages. The etiology of this difference is unclear, but may be important for understanding risk differences in the incidence of preterm labor and pre-eclampsia between black and white women.



264

Intraamniotic Infection Upregulates Vascular Endothelial Growth Factor (VEGF) in Term Decidua: Implications for Infection-Related Preterm Birth and Abruption. Victoria Snegovskikh,[1] Frederick Schatz,[*1] Felice Arcuri,[2] Paulo Toti,[2] Lynn Buchwalder,[1] Rebecca Caze,[1] Mizanur Rahman,[1] Hee Joong Lee,[1] Se-Te Joseph Huang,[1] Catalin Buhimschi,[1] Irina Buhimschi,[1] Charles Lockwood,[*1] Errol Norwitz.[*1] *[1]Obstetrics, Gynecology & Reprod Sciences, Yale University, New Haven, CT; [2]Human Pathology and Oncology, University of Siena, Sienna, Italy.*

OBJECTIVE: In early pregnancy, decidual VEGF regulates angiogenesis and is required for implantation, placentation, and fetal development. The function of decidual VEGF in later pregnancy is less well understood. This study investigates the effects of: (i) intraamniotic infection (IAI) on VEGF expression in vivo, and (ii) the immunoregulatory cytokines, IL-1b and TNFa, on VEGF production by term decidual stromal cells (DSCs) in vitro.

METHODS: (i) Immunohistochemical studies were performed on tissue sections of term decidua with or without clinical / histologic evidence of IAI (n=3 for each) using anti-VEGF antibodies (Santa Cruz, CA). VEGF expression in IAI vs controls was scored by an investigator blinded to the identity of the samples. (ii) Term DSCs were retrieved from elective cesarean (n=7), purified, and depleted of leukocytes. Confluent term DSCs Cells were treated with 10^{-8}M estradiol (E2), 10^{-7}M medroxyprogesterone acetate (P), both, or vehicle for 7 days. After 24h incubation in fresh media, cells were stimulated with IL-1b (0.01-10 ng/mL) or TNFa (1 ng/mL) for 24h. Levels of free VEGF in conditioned supernatant were measured by specific ELISA and corrected for cell protein.

RESULTS: VEGF expression in term decidua was significantly increased in tissues with evidence of IAI vs controls ($p<0.05$), and localized primarily to DSCs. IL-1b enhanced VEGF production by term DSCs in a dose-dependent fashion irrespective of the hormonal milieu (eg, 2.7-fold stimulation by 1 ng/mL IL-1b from 5.0±1.2 to 13.2±3.8 pg/mL per mg protein for E2+P; p=0.034); no stimulation was seen with TNFa (eg, 5.0±1.2 vs 5.6±1.2 pg/mL per mg protein for E2+P; p=0.89).

CONCLUSIONS: IAI is associated with increased VEGF expression in term decidual tissue in vivo. IL-1b (but not TNFa) stimulated VEGF production in term DSCs in vitro. Since aberrant VEGF expression alters vascular permeability, these data provide a mechanism by which IAI can promote extravasation into the decidua of maternal clotting factors (with thrombin generation and abruption) and leukocytes (with 'decidual activation' and preterm labor).

265

***E. coli* Induced Preterm Delivery in the Mouse Is Completely Dependent upon the Toll-Like Receptor Adaptor Protein MyD88.** Yana Filipovich,[1] Shi-Jiang Lu,[1,2] Emmet Hirsch.[*1,2] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

OBJECTIVE: Toll-like receptors (TLRs) are the critical recognition factors for initiating innate immune responses to bacterial pathogens. We have previously shown using mutant mice that TLR-4 (frequently referred to as the LPS receptor) is essential for normal susceptibility to *E. coli*-induced preterm labor. Engagement of TLR-4 by its ligand triggers activation of two independent intracellular signal transduction pathways, one known as the

'MyD88-dependent' pathway and the other as the 'MyD88-independent' (TRIF-dependent) pathway. The purpose of this study was to test whether *E. coli*-induced labor is mediated via MyD88, TRIF or both.

METHODS: TRIF knockout (n=41), MyD88/TRIF double-knockout (n=25) and B6129SF2/J wild-type control (WT, n=27) mice on day 14.5 of gestation underwent intrauterine injection with varying quantities of killed *E. coli*. Mice were observed after surgery for preterm delivery (delivery of at least one pup within 48 hours).

RESULTS: TRIF-deficient and wild-type mice were no different from each other in their dose-responsive susceptibility, while mice lacking both TRIF and MyD88 were completely protected from bacterially induced preterm delivery.

Pregnancies with preterm delivery after treatment

# organisms	5 x 10^8	10^9	5 x 10^9	10^{10}
WT	3/6 (50%)	7/9 (78%)	8/8 (100%)	4/4 (100%)
TRIF KO	5/12 (42%)	8/11 (73%)	9/11 (82%)	7/7 (100%)
MyD88/TRIF KO		0/6 (0%)	2/12 (17%)	0/7 (0%)
P value (chi square)	1.000	0.005	<0.001	<0.001

CONCLUSIONS: Susceptibility to *E. coli*-induced preterm labor in the mouse is not altered by TRIF deficiency but is completely prevented by combined MyD88/TRIF deficiency. These results suggest that of the two alternate signaling pathways downstream of TLR-4, the MyD88-dependent pathway is the critical one for bacterially induced preterm labor.

266

Preventing Fetal Brain Injury in Preterm Birth: Is Targeting TLR-4 the Answer? Michal A Elovitz,[*1] Jinghua Chai,[1] Juan Gonzalez,[1] Jean Richa.[2] *[1]Dept. OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Genetics, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: An increase in adverse neurological outcomes has been observed in neonates from preterm births (PTB) complicated by inflammation. One hypothesis is that inflammation-induced neonatal brain injury is the result of bacterial by-products, such as lipopolysaccharide (LPS), reaching the fetus and activating the LPS receptor, TLR-4. These studies were performed to determine if activation of TLR-4 in the fetal brain is an essential mediator of fetal brain injury in inflammation-induced PTB.

STUDY DESIGN: An established mouse model of PTB and embryo transfer technique was used. Timed pregnant CD-1 dams; CD-1 dams with embryo transfered CD pups; CD-1 dams with HEJ (TLR-4 -/-) pups; and CD-1 dams with OUJ (WT to HEJ) were randomized to intrauterine injection with saline (NS) or LPS into the lower right horn. 6 hrs after infusion, placentas, and fetal brains were collected from the upper left horn (3-5 pups per dam; 6-9 dams per treatment group). Amniotic fluid (AF) was collected for LAL assay to determine the time period for intrauterine LPS to reach AF. Expression of inflammatory mediators and markers of glial development were assessed by quantitative PCR.

RESULTS: LPS is present in AF in the upper left horn by 3 hrs. SEE FIGURE. Embryo transfer blunted the LPS-induced cytokine response in CD fetal brains. IL-1, TNF and CCL3 expression were similarly affected by LPS between OUJ and HEJ brains. IL-13 mRNA was increased 5.5-fold in HEJ brains compared to OUJ (P<0.001). Fold changes(LPS/NS) in GFAP, PLP, MAG and MBP mRNA were not significantly different between OUJ and HEJ fetal brains. Nestin and NCAM were significantly increased in OUJ brains exposed to LPS; this up-regulation was not observed in HEJ fetal brains.

CONCLUSION: TLR-4 modulates the fetal/placental immune response to inflammation-induced PTB. However, if a placental or fetal brain cytokine response is mechanistically involved in neonatal brain injury, then targeting TLR-4 is unlikely to be effective at preventing adverse neurological outcomes from inflammation-induced PTB.

	Fold difference in target gene expression between placentas from LPS exposed dams compared to Saline Exposed Dams		
Inflammatory Mediator	CD	OUJ	HEJ
IL-1β	4.8	4.0	4.2
IL-10	10.9	5.3	6.5
IL-13	1.6	0.8	0.2
TNF	6.9	5.2	8.1
IL-6	10	5.8	8.2
CCL2	7.5	2.4	0.6
CCL3	6.9	10.7	4.5
CCL5	6.5	12.8	2.9
CCL11	1.3	0.7	0.7

267

Diagnostic Markers for Early Detection of Ovarian Cancer. Gil Mor,[*1] Irene Visintin,[1] Yinglei Lai,[1] Thomas Rutherford,[1] Peter Schwartz,[*1] David Ward,[1] Ayesha B Alvero. *[1]Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA; [2]The Nevada Cancer Institute, Las Vegas, NV, USA.*

Background: Ovarian Cancer (OC) is the fourth leading cause of cancer-related death in women in the USA and the leading cause of gynecologic cancer death. OC is characterized by few early symptoms, presentation at an advanced stage, and poor survival. The high mortality rate is due to the difficulties with the early detection of ovarian cancer. Indeed, approximately 80% of patients are diagnosed with advanced staged disease. Early detection would significantly decrease the mortality of ovarian cancer. In this study we characterize and validate the combination of six serum biomarkers using an innovative Multiplex system that discriminates between disease-free and ovarian cancer (OC) patients with high efficiency.

Methods: Serum from 363 healthy controls and 156 newly diagnosed OC patients were analyzed. Concentrations of leptin, prolactin, osteopontin, insulin-like growth factor-II, macrophage inhibitory factor, and CA125 were determined by Multiplex technology. All 6 markers were evaluated in: 1) Training set: 74 samples from the control group and 74 samples from patients with ovarian cancer. 2) Validation set: 289 samples control group and 82 ovarian cancer.

Results: In the training set, none of the biomarkers by themselves were good enough to differentiate healthy vs cancer. However, the combination of the six markers provided a better differentiation of the two groups. The final results combining both training and validation groups were: from 363 healthy samples, one was misclassified, specificity 99.67%. From 156 OC samples, 3 were misclassified, sensitivity 97.5%.

Conclusion: We describe the first blood biomarker test with a specificity of 99.7 % for the detection of ovarian cancer. Six markers provided a significant improvement to the sensitivity and specificity for ovarian cancer detection. Validation of the test was performed with a blinded cohort. This novel Multiplex platform has the potential for efficient screening in high-risk patients for ovarian cancer.

268

Characterization of the Human HOXA10 Gene Promotor and Transcriptional Repression by the Wilms' Tumor Suppressor Gene Product (WT1). Vaagn Andikyan, Hongling Du, Hugh S Taylor.[*] *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: Homeobox genes encode transcription factors that control cell differentiation and patterning. Increasing evidence indicates that many homeobox genes are aberrantly expressed in cancers, and that their dysregulation contributes to tumor progression. The homeobox gene HOXA10 controls uterine organogenesis during embryonic development and functional endometrial differentiation in the adult. This study identifies a novel, functional 5' repressor element of HOXA10.

Methods: To identify regulatory activity, we cloned 2 KB 5' of the HOXA10 transcription start site. Nested deletion were created, cloned into pGL3 reporter constructs and used in luciferase assays using Ishikawa and human endometrial stromal cell lines (HESC). Renilla luciferase activity was used for normalization. Transfection was performed with Lipofectamine in Ishikawa and Mirus in HESC. Transcription factor binding sites were identified using MatInspector. Mutagenesis was performed using site directed mutagenesis. Student's t-test was used for statistical evaluation.

Results: A segment -965/-33 bp 5' of HOXA10 minimally drove reporter gene expression, while a focal element within this region located -965/-563 (P2) upstream region of promoter did drive expression; these results suggested the existence of a repressor element in the -563/33 region. In Ishikawa cells P2 drove luciferase reporter gene activity to 5 times that of empty pGL3 (p<0.001). Addition of the repressor element to P2 drove reporter activity to -5 times that of P2 alone(p< 0.0001), returning to near basil levels. In HESC transfection with P2 resulted in reporter activity similar to pGL3. We identified 2 binding sites for Wilms tumor supressor (WT1) in the repressor region. The site directed mutagenesis of WT1 binding sites confirmed that WT1 directly binds and represses reporter gene expression.

Conclusion: We demonstrate a WT1 regulated suppressor region located between -562 and -33 upstream of the HOXA 10 gene. WT1 is a known transcriptional regulator, controlling the expression of genes involved in proliferation and differentiation. Absence of this factor causes urogenital developmental abnormalities and cancer, including Wilms tumor, accompanied by high levels of expression several homeobox genes. These findings suggest a novel regulatory relationship in which homeobox genes are directly regulated by WT1.

269

Indolamine 2, 3-Dioxygenase: A Potential Mechanism of Tolerance in Human Ovarian Cancer. Jeannine A Villella,[1] Chris Andrews,[2] Eiichi Sato,[3] Peter J Frederick,[1] Richard Cheney,[1] Luc Pilotte,[3] Shashi B Lele,[1] Feng Qian,[1] Kunle Odunsi.[*1,3] *[1]Div. of Gyn Onc, Roswell Park Cancer Institute, Buffalo, NY, USA; [2]Dept of Biostatistics, SUNY at Buffalo, Buffalo, NY, USA; [3]Ludwig Institute for Cancer Research, New York, NY, USA.*

Purpose:
Indolamine 2, 3-dioxygenase (IDO) was previously identified as promoting immune tolerance and to promote induction of regulatory T cells via activity of tolerogenic dendritic cells. We hypothesize that IDO may play a mechanistic role of immune tolerance in ovarian cancer.

Experimental Design:
We performed immunohistochemical analysis for IDO and tumor infiltrating lymphocyte (TILs) in 108 cases of ovarian cancer. IDO expression, intensity and staining patterns (nuclear, cytoplasmic) were recorded. The interrelationship between TILs and expression of IDO was investigated. These results were then analyzed for statistical relationship with demographic data. Survival distributions were calculated by the Cox's proportional hazards model or Kaplan-Meier's product limit estimator. In either case, curves were compared across groups using the log rank test. Due to skewness, the number of TILS was compared across groups with Kruskal-Wallace rank sum procedures rather than ANOVA. To correlate the immunohistochemical IDO results with gene expression, we randomly selected a group of tumors and performed real time PCR to quantify gene expression of IDO. We then correlated this value to the TILs results to inquire if a relationship exists between relative gene expression of IDO and TILs in the tumor microenvironment.

Results:
The median age was 62 years, 74% had stage IIIC disease and 90% had grade 3 histology. Seventy-four (69%) of patients have died, and the median time to death is 3.3 years (CI 2.5, 4.1). The estimated median time to progression is 2.7 years. IDO expression or staining patterns did not correlate with survival, histology or stage. All specimens had cytoplasmic staining, while 19 (5.7%) also had positive nuclear staining. The strongest relationship was between TILs and nuclear staining pattern of IDO approached statistical significance (p=.07).

Conclusion:
IDO demonstrated high frequency expression in ovarian cancer patients and is suggestive of a potential mechanism for tolerance induction. The nuclear immunohistochemical staining pattern of IDO may indicate increased enzyme activity and nuclear staining pattern correlates with TILS.

270

Regulation of FOXO1A Expression in Endometrial Carcinoma. Erin C Ward, Anna V Hoekstra, John R Lurain, Diljeet K Singh, Barbara M Buttin, Julian C Schink, J Julie Kim.[*] *Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Objective: In 30-80% of Type I endometrial cancers, the PTEN gene is inactivated. The loss of PTEN activity leads to constitutively active Akt, which inhibits several downstream targets including FOXO1A. FOXO1A is a member of the FOXO sub-family of Forkhead/winged helix family of transcription factors that is involved in cell cycle regulation, differentiation and apoptosis, and is highly expressed in the human endometrium. We propose that the deregulation of FOXO1A protein in the endometrium results in the aberrant expression of genes that regulate the cell cycle and apoptosis, thereby promoting tumorigenesis.

Methods: Immunohistochemical staining for FOXO1A was done for normal and endometrial cancer tissues. RT-PCR and western blot analyses were conducted on 4 endometrial cancer cell lines, Ecc1, Ishikawa, Hec1B and RL95 to look at FOXO1A mRNA and protein expression levels respectively. To begin elucidating the mechanism of FOXO1A regulation, western blot analysis was used to determine levels of Skp2, the oncogenic subunit of the Skp1/Cul/F-box protein ubiquitination complex, in the four cell lines. In two independent experiments, siRNA techniques were used to silence Skp2 in Ishikawa cells and an inhibitor of Akt was applied to the same cell line to inhibit phosphorylation of FOXO1A. Western blot analyses were used to ascertain the effects of the two treatments on FOXO1A protein expression. Immunofluorescence for FOXO1A was conducted on Akt inhibitor treated Ishikawa cells to determine the localization of FOXO1A.

Results: In normal endometrium, FOXO1A protein was highly expressed in both the glands and stroma while endometrial carcinoma showed very little to no detectable staining for FOXO1A. The four endometrial carcinoma cell lines demonstrated that FOXO1A mRNA was expressed while very little FOXO1A protein was present. Skp2 was expressed in all four cell lines with the highest levels produced by the Ishikawa cells. Levels of FOXO1A protein increased when Ishikawa cells were treated with a siRNA specific to Skp2. Finally, FOXO1A protein levels increased and the protein was localized to the nucleus in Ishikawa cells after treatment with an Akt inhibitor.

Conclusions: The loss of FOXO1A may be an important event in endometrial neoplasia and determining the mechanisms to reinstate this pathway will provide insight to the development of alternate therapies.

271

Expression Analysis of the VEGF Receptor Family in Mouse Peri-Implantation Uterine Vessel Formation. Nataki C Douglas, Hongyan Tang, Raul Gomez, Mark V Sauer,[*] Ralf C Zimmermann.[*] *Obstetrics and Gynecology, Columbia University, New York, NY, USA.*

Objective: Prior to placenta formation, interactions between the embryo, endometrium and ovary create a uterine environment that supports early embryonic development. Induction of angiogenesis in the uterine decidua is a key event in peri-implantation uterine differentiation and blastocyst implantation. Members of the VEGF receptor family are major regulators of angiogenesis and lymphangiogenesis. To determine the factors involved in peri-implantation uterine vessel formation, we examined the expression of VEGFR-1, VEGFR-2, VEGFR-3, PECAM (blood endothelial cell marker), and LYVE-1 (lymphatic endothelial cell marker) on embryonic days (EDs) 4.5 to 7.5.

Design: Expression analysis

Materials and Methods: 8 week old mature female CD1 mice were mated with males of proven fertility. A vaginal plug the following morning was interpreted as successful mating and counted as day 0.5 of pregnancy. Mice (n=16, 4 mice per ED) were sacrificed on EDs 4.5, 5.5, 6.5 and 7.5. Cross sections of uteri were processed for immunohistochemical analyses with the following antibodies: PECAM, LYVE-1, VEGFR-1, VEGFR-2, and VEGFR-3. Immunofluorescent double staining was performed according to standard protocols.

Results: Blood vessels are present throughout the peri-implantation uterus. Vascular density starts to increase on ED 4.5, the time of implantation, peaks on ED 5.5-6.5, and declines on ED 7.5 at the site of placenta formation. Lymphatic vessels are present around the embryo, in the primary decidual zone (PDZ). Double staining of LYVE-1 and PECAM confirms unique populations of blood and lymphatic vessels.

Whereas, VEGFR-2 is expressed on vessels of the PDZ and secondary decidual zone (SDZ), VEGFR-1 expression is restricted to the PDZ, and VEGFR-3 expression is restricted to vessels in the mesometrial pole of the PDZ. Double staining to detect co-localization of PECAM with VEGFR-1 or VEGFR-2 indicates that these receptors are expressed on blood endothelial cells. Double staining to detect co-localization of VEGFR-3 with PECAM or LVYE-1 indicates that VEGFR-3 is expressed on both blood and lymphatic vessels.

Conclusions: Peri-implantation uterine vessel formation involves both angiogenesis and lymphangiogenesis. The differential expression pattern of VEGFR-1, VEGFR-2, and VEGFR-3 in the peri-implantation uterus suggests that each VEGF receptor plays a unique role in blood and/or lymphatic vessel formation.

272

Ovarian Cancer Cell Attachment to Extracellular Matrix Is Regulated by S1P and LPA. Yoel Y Smicun, Orlando Gil, Kate Devine, Jennifer Gilman, David A Fishman.[*] *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*

Objective: Lysophophatidic acid (LPA) and sphingosine-1-phosphate (S1P) are found in high levels in serum and ascites of ovarian cancer patients. We have demonstrated that LPA and S1P regulate invasion of epithelial ovarian carcinoma (EOC) cells. Detachment and reattachment to extracellular matrix (ECM) are integral to invasion. Therefore, we investigated effects of S1P and LPA on cell-ECM attachment, cell-ECM adhesion protein β1-integrin and membrane associated focal adhesion kinase (FAK), which connects ECM-integrin complexes to the cytoskeleton.

Methods: After 4 hrs treatment with 40μM LPA, 0.5μM S1P or 20μM S1P, Dov13 EOC cells were labeled with calcein and applied to Matrigel- or collagen-I-coated wells, incubated, washed, and counted. Attached cells were lysed or detached and allowed to reattach for 3, 6 or 24 hrs (simulating invasion) before being lysed. Membrane and cytoplasm fractions were analyzed by Western blot.
Results: Pretreatment with 40μM LPA decreased attachment to Matrigel by 2/3 (p=0.031) but did not significantly affect attachment to collagen-I. Both S1P concentrations increased attachment to Matrigel (1.5fold)(p=0.045,0.009) and collagen-I (3 fold)(p=0.015,0.005). Cell-matrix adhesion protein β1-integrin and membrane-associated FAK increased dose-dependently in attached cells pretreated with S1P but decreased with LPA pretreatment, in correlation with the phospholipids' respective effects on attachment. FAK was depleted from membranes of reattaching cells regardless of treatment. Treatment during attachment with LPA, S1P, Lyso-phosphatidylcholine (LPC) and lyso-phosphoglycerol (LPG) (phospholipids without know biological activity) was inhibitory.
Conclusions: ECM attachment contributes to cell invasion, and LPA and S1P appear to regulate both processes. While 40μM LPA pretreatment significantly increased cell invasion and decreased Matrigel attachment, 20μM S1P stimulated attachment to Matrigel and inhibited invasion. Interestingly, 0.5μM S1P increased both attachment and invasion, indicating invasion is a complex process not entirely dependent on decreased attachment. All tested phospholipids were inhibitory when applied during attachment, suggesting that S1P and LPA act via different mechanisms depending upon whether EOC cells are attached. Focal adhesion is likely a negative regulator of invasion as FAK was depleted from membrane of attaching cells.

273

2-Methoxyestradiol & Multidrug Resistance: Can 2-Methoxyestradiol Sensitize Resistant Breast Cancer Cells? Samar Azab,[1] Salama Salama,[1] Ashraf Abdel-Naim,[2] Amani Khalifa,[2] Ebtehal El-Demerdash,[2] Ayman Al-Hendy.*[1] *[1]Ob/Gyn, UTMB, TX, USA; [2]Pharmacology & Toxicology, ASU, Egypt.*
Background
Development of resistance to anticancer agents represents a major impediment to successful treatment of breast cancer. Thus, searching for compounds able to modulate multidrug resistance (MDR) & have low in vivo toxicity is an important goal. 2-methoxyestradiol (2ME), a natural derivative of estradiol possessing antiproliferative effect, is currently evaluated in phase 1 & 2 clinical trials for advanced solid tumors *(Cancer Research, 2005, v.65,2:387).*
Objectives
This study aims to evaluate the modulatory effects of 2ME on regulation of MDR & cytotoxicity of doxorubicin (Dox).
Methods
The effects of Dox alone & in combination with 2ME were tested in Dox resistant breast cancer cells MCF-7/Dox. Cytotoxicity was addressed by MTT assay. In addition, 3 pathways regulating MDR were investigated. 1st MDR1 gene was chosen as a representative of the drug resistance pathway. 2nd Bcl 2 & P53 were selected for the apoptotic pathway. 3rd Cyclin D1 was tested for the cell cycle regulatory pathway. The transcription & translation of these genes were tested using RT Profiler PCR Array & western blotting. Lastly, the function of the 3 pathways were studied using p-glycoprotein (p-gp) function, caspase 3 activity & flowcytometric cell cycle assays.
Results
2ME increased the sensitivity of the resistant MCF-7/Dox cells to the cytotoxic effect of Dox by 2.9 folds (IC_{50} for Dox alone was 262±54 μM & for Dox in combination with 100nM 2ME was 91±13 μM, p<0.05). Combination of 2ME & Dox altered significantly genes responsible for MDR regulation. Addition of 2ME to Dox was found to decrease Bcl 2 & Cyclin D1 expression. On the other hand, MDR1 was over expressed while P53 expression was not affected by combination treatment. Array results were confirmed with western blots. Furthermore, 2ME increased p-gp function by 24 ± 7.1%, compared to control. Addition of 2ME to Dox treatment increased Caspase 3 activity by 2 folds. Finally, combination of 2ME & Dox induced an arrest of the cell cycle in S phase (25±0.7%) as compared to Dox alone (20±1%, p<0.01).
Conclusions
2ME sensitizes resistant breast cancer cells to Dox cytotoxicity. This sensitization can be explained by down regulating Bcl 2 & Cyclin D1 expression, augmenting the caspase 3 activity & inducing a cell cycle block in the S phase. These results explore the genetic events mediating the synergistic effect of Dox & 2ME.

274

Vascular Endothelial Growth Factor (VEGF) Autocrine Signaling Induced Proliferation of Endometrial Cancer Cells. Takehiro Serikawa,[1] Daniel C Chung,[2] Maureen P Lynch,[1] John S Davis,[3] Ruben R Gonzalez,[1] Bo R Rueda.*[1,4] *[1]Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA, USA; [2]Department of Gastroenterology, Massachusetts General Hospital, Boston, MA, USA; [3]Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE, USA; [4]Microbiology, Biochemistry & Immunology, Morehouse School of Medicine, Atlanta, GA, USA.*
Background: VEGF plays an important role in the neovascularization of malignant gynecological tissues and has been used as a prognostic marker for multiple tumor types including gynecological.
Objectives: Our objectives were to 1) compare VEGF levels in benign and malignant endometrial epithelial cells, 2) define the signaling pathways responsible for upregulation of VEGF in endometrial cancer cells (EnCa), and 3) determine if VEGF has autocrine/paracrine effects on EnCa cell proliferation.
Methods: Levels of VEGF mRNA in EnCa cell lines (RL-95, HEC1A, HEC1B, SK-UT2, Ishikawa, AN3CA) and short-term cultured primary endometrial epithelial cells (EEC, n=3) were determined by Q-PCR. VEGF protein was assessed in conditioned medium by ELISA. VEGF promoter activity was evaluated by luciferase reporter assay. EnCa cells were treated with the following inhibitors: PI3K/AKT/mTOR (Adeno-PTEN, Wortmannin, Rapamycin), JAK pathway (AG490), and MEK1/ERK 1/2 pathways (PD098059) to assess their contribution to the regulation of VEGF. Cell proliferation was determined by cell counts.
Results: Benign EEC expressed less VEGF (p<.001) when compared to the high level expressed in 5 of 6 EnCa cells The increase in protein was not concordant with VEGF mRNA expression in all EnCa cell lines. In addition, there was no difference in VEGF promoter reporter activity in the benign and malignant cells. Chemical inhibition of the PI3K pathway reduced but did not completely inhibit VEGF secretion by An3Ca cells (p < .001). Adeno-PTEN or PD098059 only modestly reduced VEGF production (p < .05 each). In contrast, inhibition of JAK2 increased (p < .05) levels of VEGF without an increase in HIF1α. Inhibition of VEGF receptor activation with Adeno sFLT reduced (p < .05) EnCa cell proliferation.
Conclusions: EnCa cells produce significantly more VEGF than their benign counterparts. Multiple signaling pathways contribute to VEGF production/secretion in EnCa cells. VEGF produced by EnCa cells has autocrine/paracrine effects on cell proliferation.

275

Differential Insulin Receptor Splicing in Trophoblast vs Endothelial Cells of the Human Placenta and Splicing Dysregulation in Gestational Diabetes but Not in IUGR. Gernot Desoye,*[1] Tatjana Radaelli,[2] Gioia Alvino,[2] Christian Wadsack,[1] Irene Cetin,*[2] Ursula Hiden.[1] *[1]Dept Obstetrics & Gynecology, Medical University of Graz, Graz, Austria; [2]Inst Obstetrics & Gynecology, Foundation IRCCS PoMaRe, University of Milano, Milano, Italy.*
BACKGROUND AND OBJECTIVES:
Human first trimester (FT) and term (TT) trophoblast and endothelial cells (EC) express insulin receptors (IR). Two IR isoforms differ by inclusion of exon 11 (Ex11) and by signaling efficiency. Alternative splicing is accomplished by splicing factors of the MNBL and CELF (CUGBPs) family favoring the longer (Ex11+) and shorter (Ex11-) isoforms, respectively. This study hypothesized differential IR splicing and, hence, insulin signaling in FT/TT vs EC as a result of tissue-specific expression of splicing factors, and dysregulation of splicing in pathological pregnancies.
METHODS:
IR isoforms and splicing factors were measured (RT-PCR) in primary placental cells (EC, FT, TT) and cell lines and in placentas from normal, gestational diabetic (GDM) and IUGR pregnancies. Cells were cultured under various insulin, glucose and dexamethasone concentrations. IR splicing effect on signaling was determined by measurement of ERK1/2 and PKB phosphorylation (western blot) after insulin stimulation (0-60 min).
RESULTS:
IR splicing differed in trophoblast vs endothelial cells: FT/TT expressed IR11+ (>90%), whereas EC mainly expressed IR11- (70%). Splicing factor MBNL3, promoting Ex11 inclusion, prevailed in IR11+ producing FT/TT, whereas CUGBP2, supporting Ex11 skipping, prevailed in IR11- expressing EC. Mitogenic insulin signaling differed in FT/TT vs EC. Splicing was unaltered in vitro by glucose, insulin, and dexamethasone. IR11- expression was lower

($p<0.05$) in both IUGR and GDM than in normal placentas. The changes in IUGR were the result of altered cellular composition of the placentas whereas abnormal IR splicing accounted for the changes in GDM.

CONCLUSION:

Cell type specific splicing of IR in the human placenta accomplished by differential expression of splicing factors allows maternal vs fetal insulin to regulate placental function separated in space with a preferential activation of mitogenesis in trophoblasts. Reduced IR11- expression in GDM highlights the role of proper IR splicing/insulin signaling for placental development.

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276

Growth Factor Stimulation of Protein Synthesis in Trophoblast Cells Is Mediated by the mTOR Signaling Pathway. Nicholas P Illsley,* Stacy Zamudio,* Andrea Albieri. *Obstetrics, Gynecology and Women's Health, UMDNJ-New Jersey Medical School, Newark, NJ, USA.*

Introduction: The mTOR (mammalian Target Of Rapamycin) signaling pathway is responsible for integrating the effects of nutrients and growth factors in the regulation of major functions involved in cellular growth. Foremost of these is the control of mRNA translation, however little is known about the processes regulating protein synthesis in trophoblast cells. As protein synthesis plays a crucial role in placental growth, we designed this study to investigate whether mTOR is involved in the regulation of protein synthesis in trophoblast cells. The specific inhibitor, rapamycin, was used to identify mTOR modulated processes. We hypothesized that inhibition of the mTOR signaling pathway would be associated with a decrease in the extent and efficiency of protein synthesis. **Methods:** BeWo choriocarcinoma cells were incubated for 24 hr in DMEM/10% FBS in the presence and absence of 0.2µM rapamycin. Extracted cells were blotted for phospho- and total mTOR, p70S6 kinase 1 (p70S6K1) and 4E-binding protein 1 (4E-BP1) and quantitated by densitometry. Cell culture lysates, after removal of the nuclear fraction, were separated on a 15-50% sucrose gradient and fractionated to generate an RNA profile by measuring optical density at 254 nm. The poly(ribo)somal section of the profile was integrated to quantify the extent and efficiency of translation. **Results:** Rapamycin did not inhibit mTOR phosphorylation but it reduced phosphorylation of p70S6K1 and 4E-BP1, to 23 ± 7% and 67 ± 15% of the FBS-treated control ($p < 0.05$, n=4). After fractionation, integration of the polysomal faction revealed that RNA associated with the polysomes was reduced to 65 ± 7% of control by rapamycin ($p < 0.05$, n=4). Translational efficiency in the same samples was reduced to 71 ± 9% of control by inclusion of rapamycin. **Conclusions:** Both p70S6K1 and 4E-BP1, located downstream of mTOR, are involved in the initiation of protein synthesis. Rapamycin inhibited the phosphorylation of p70S6K1 and 4E-BP1, indicating that the phosphorylation process is mediated by mTOR kinase. Rapamycin also caused a decrease in the extent and efficiency of protein synthesis. We conclude that regulation of mRNA translation in trophoblast cells is mediated, at least in part, via the mTOR signaling pathway. *(Supported by NIH HD046982 to NPI).*

277

Evidence for Syncytin Expression in Human Placental Exosomes. Jorge M Tolosa,[1,2] John E Schjenken,[1] Vicki L Clifton,[*1] Roger Smith.[*1] *[1]MBRC, University of Newcastle, Newcastle, NSW, Australia; [2]Faculty of Medical Sciences, University of Santiago of Chile, Santiago, Metropolitan Region, Chile.*

Background: Syncytin, a human endogenous retrovirus envelope protein encoded by the human endogenous retrovirus (HERV-W), is expressed in the placenta and has fusogenic properties as well as a putative immunosuppressive peptide sequence (ISU) within the transmembrane (TM) subunit of the protein. Syncytin is thought to play a critical role in human placental morphogenesis by mediating cytotrophoblast fusion to form the syncytiotrophoblast. Exosomes are membrane vesicles that are released by the cell upon fusion of multivesicular bodies with the plasma membrane. Placental exosomes are immunosuppressive and retroviruses may use the exosomal pathway to propagate. We hypothesise that the human placenta produces exosomes that carry the retroviral protein syncytin.

Methods: We have raised antibodies to the putative immunosuppressive peptide of syncytin (ISU). Human placental explants were cultured for 72 hours at 37°C (5% CO2). Following culture, the supernatant was removed and exosomes were enriched by differential ultracentrifugation (pelleting) and/or sucrose gradient fractionation (rate zonal separations). Proteins were extracted from the exosomal fraction using a CHAPS based buffer, SDS-PAGE Silver staining and Western Immunoblotting were used to analyse the proteins. The presence of exosomes was confirmed by Transmission Electron Microscopy.

Results: Exosomes were isolated using three different procedures; differential centrifugation, differential centrifugation followed by sucrose gradient and differential centrifugation using a sucrose cushion followed by sucrose gradient. Differential centrifugation with a sucrose cushion produced a highly purified exosomal fraction. Using Transmission Electron Microscopy (TEM), 100 nm membrane vesicles were observed; the predicted size and density of exosomes. Western Blotting analysis of the enriched placental exosome fractions identified a 24kDa protein, the expected size of the Syncytin TM subunit. Preliminary observations show cross-reactivity of our antibody with a 24kDa protein present in human plasma from pregnant women.

Conclusions: Human placental exosomes express a protein with the molecular weight and immunological characteristics of the 24kDa protein corresponding to the TM subunit of syncytin. These results suggest a novel mechanism by which the placenta may influence the maternal immunology during pregnancy.

278

Placental Villous Trophoblast Morphology in Early- and Late-Onset Pre-Eclampsia (PET) with and without IntraUterine Growth Restriction (IUGR). Kate Widdows,[1] Michael Egbor,[1] Paul Sibbons,[1] John Kingdom,[*2] Tahera Ansari.[1] *[1]Department of Surgical Research, Northwick Park Institute for Medical Research, Harrow/London, United Kingdom; [2]Department of Obs & Gyn, Mount Sinai Hosptial, University of Toronto, Toronto, Canada.*

Objective The villous trophoblast is the biological machinery of the human placenta responsible for maternofetal exchange. Alterations in the morphology of this membrane may lead to perturbed oxygen and nutrient transfer ultimately leading to compromised fetal growth. PET and IUGR are associated with alterations in placental villous and vasculature morphology and alterations specific to the trophoblast are yet to be described. The objective of this study is to stereologically assess morphological alterations in placental villous trophoblast in placentae from pregnancies complicated by PET, with and without associated IUGR, and to determine what effect age-of-onset has on trophoblast morphology and whether these changes, if any, have detrimental effects on villous membrane function.

Methods 39 placentae were collected from pregnancies complicated by PET (n=9), IUGR (n=10), PET associated with IUGR (n=10), and gestational-aged-matched controls (n=10). Placentae were divided into early-onset (< 34 weeks gestation) or late-onset cases (>34 weeks) for each study group. Placental samples were obtained uniform-randomly and 5µm formalin-fixed paraffin embedded sections were stained with H&E. Trophoblast morphology was assessed stereologically and volume estimates were obtained for cytotrophoblast, syncytiotrophoblast and syncytial knots.

Results Placentae from early-onset PET were associated with significant reductions in the volume of the cytotrophoblast, syncytiotrophoblast, syncytial knots and total placental volume (all $p=<0.001$) when compared to age-matched controls. Neglible effects on trophoblast morphology were observed in placentae from late-onset PET with significant reductions observed in the volume of syncytial knots ($p=0.041$). Early-onset IUGR had no effect on trophoblast morphology, whereas placentae from late-onset IUGR were associated with significant reductions in syncytiotrophoblast volume ($p=0.004$) and total trophoblast volume ($p=0.003$).

Conclusion Alterations in trophoblast morphology are dependent upon age-of-onset in PET but not in IUGR further validating the hypothesis that PET can be classified as two separate disease entities, one with abnormal placental pathology and one with normal placental pathology.

279

Elevation of Placental 11βHSD2 Activity at High Altitude and Fetal Sex Specific Decreases in Preeclampsia. Stacy Zamudio,[*1] Michael Stark,[2] Nicholas Illsley,[*1] Tatiana Torricos,[3] Tatiana Alvarez,[3] Enrique Vargas,[3] Vicki Clifton.[*2] *[1]Ob/Gyn & Women's Health, NJ Med. Sch, Newark, NJ, USA; [2]Mothers & Babies Res. Ctr, John Hunter Hosp, Newcastle, NSW, Australia; [3]Inst. Boliviano Biología Altura, La Paz, Bolivia.*

Introduction: Residence at high altitude (>2700 m) increases the incidence of preeclampsia and decreases birth weight. Placental 11β-HSD2 activity is positively correlated with birth weight, suggesting that the activity of this enzyme is important in fetal growth. We have shown that placental 11β-HSD2 expression and activity are fetal sex-specific. In the male fetus under maternal stress 11βHSD2 activity is maintained, and male fetuses maintain normal growth. Expression and activity are down-regulated in female fetuses, as

is growth, under similar levels of maternal stress. We asked whether ethnic differences in growth under hypoxic stress are associated with differences in 11β-HSD2 activity in the placenta, and whether there are sex-specific changes in activity in preeclampsia (PE).
Methods: TLC was used to evaluate placental 11β-HSD2 activity in women of European ancestry (n=18 at 300 m, 24 at 3600 m) vs. Andean ancestry (n=18 at 300 m, 27 at 3600 m), and in 10 Andean women with PE at 3600 m.
Results: Altitude increased placental 11β-HSD2 activity in the placentas of European migrants to high altitude, but not in natives. Increase in activity was equivalent for male vs. female fetuses among migrants to 3600 m, but did not contribute to increased fetal growth as birth weight diminished by 420 g, while similar Andean babies had only a 180 gram decrease relative to low altitude controls. In contrast to our hypothesis, and in the absence of sex-specific differences in fetal growth, placentas of Andean male fetuses had lower 11β-HSD2 activity at high vs. low altitude. Consistent with our hypothesis, preeclamptic pregnancies where fetal sex was female showed markedly down-regulated 11β-HSD2 activity, while activity was unchanged in the placentas of male fetuses.
Conclusions: We provide the first estimates of male versus female human placental 11β-HSD2 activity under conditions of mild chronic hypoxemia in low vs. high altitude pregnancies and in PE. Changes in 11β-HSD2 activity appear to be unrelated to birth weight at high altitude and suggest that changes in bioactive cortisol may not be the most influential factor on fetal growth under conditions of hypoxaemia and in the absence of PE. *Support: NIH HD42737, NSF BCS 0309142.*

280

Local Delivery of VEGF to the Uterine Arteries Increases Vessel Relaxation and Placental Perfusion in the Pregnant Sheep. Anna L David,[1] Belen Torondel,[2] Ian Zachary,[2] Manfred J Ramirez,[2] Suzanne M Buckley,[3] Terry Cook,[4] Michael Boyd,[5] Charles H Rodeck,[1] John Martin,[2] Donald Peebles.*[1]
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Hypothesis: Impaired materno-placental perfusion can lead to pre-eclampsia and fetal growth restriction. We hypothesised that local over-expression of VEGF in the uterine artery would lead to a sustained increase in uterine artery blood flow (UABF) through enhanced vasodilatation of the uterine vascular tree.
Methods: We injected adenovirus vectors containing the VEGF or β-galactosidase gene (Ad.VEGF-A or Ad.lacZ, 5 x ^{11} particles) into the left and right uterine artery of five Romney ewes at laparotomy (97 - 102 days gestation, term = 145 days); the investigators were blind to the side of VEGF injection. Using Doppler sonography, UABF was calculated as the product of blood flow and vessel cross sectional area, measured before and 4 –7 days after injection. At post mortem examination, sections were taken from the uterine arteries at 4 levels and studied in an organ bath.
Results: The mean UABF significantly increased after Ad.VEGF-A injection when compared with before injection (408 ml/min ± SD 273 to 1321 ml/min ± SD 728, p = 0.005); Ad.lacZ injection did not signficantly increase UABF. Compared with Ad.lacZ vessels, Ad.VEGF-A transduced vessels were significantly less responsive to phenylephrine (E_{max} 148±10.9 vs E_{max} 228.2±27.5, p < 0.05) and had significantly increased relaxation to bradykinin (pD2 (-log EC50) values 9.11±0.01 vs 8.65±0.11, p< 0.05). β-galactosidase expression was shown using X-gal histochemistry, β-galactosidase ELISA and immunohistochemistry.
Conclusions: Adenovirus mediated local over-expression of VEGF results in increased uterine artery blood flow and relaxation of the uterine arteries. These results suggest it may be possible to develop a therapeutic intervention to increase placental perfusion by increasing VEGF expression, to improve the outcome of pregnancies complicated by severe fetal growth restriction and pre-eclampsia.

281

Endoglin, sFlt-1, and PlGF Production by Placental Trophoblasts from Normal and Preeclamptic Pregnancies Cultured under Normoxic and Hypoxic Conditions. Yang Gu, Yanping Zhang, David F Lewis, Yuping Wang.*
Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.
Objective: Endoglin, sFlt-1, and PlGF have been considered to be biomarkers for preeclampsia. The purpose of this study is to determine if there are differences in placental trophoblast (TC) production of endoglin, sFlt-1, and PlGF between normal and preeclamptic (PE) pregnancies, and whether productions of these soluble factors are altered when cells are cultured under hypoxic condition.
Methods: Placentas were obtained from 7 normal and 7 PE pregnancies immediately after delivery. TCs were isolated and cultured with DMEM containing 2% FBS under normoxic (5%CO2/air) and hypoxic (2%O2/5%CO2/93%N2) conditions for 48hrs. Medium concentrations of endoglin, sFlt-1, and PlGF were measured by ELISA. Fresh placental tissue pieces were also snap frozen with liquid nitrogen. Total protein was extracted. Protein expressions for endoglin, sFlt-1, and PlGF in the placental tissues and in the culture media were examined by Western blot analysis. Data are presented as mean ± SE and analyzed by Mann-Whitney test and paired t-test. A p level < 0.05 is considered statistically different.
Results: 1) under normoxic condition, TCs from PE placentas produced significantly more endoglin, sFlt-1 and PlGF compared to those from normal TCs (endoglin: 1.726±0.272 vs. 1.123±0.194 pg/ug protein; sFlt-1: 0.138±0.026 vs. 0.077± 0.009 pg/ug protein; PlGF: 0.084±0.025 vs. 0.021±0.004 pg/ug protein), p < 0.05; 2) Under hypoxic condition, TCs from PE placentas, but not from normal placenta, released more endoglin and sFlt-1. In contrast, TCs from both normal and PE placentas released less PlGF; 3) Differential expressions of endoglin, sFlt-1 and PlGF between normal and PE placental tissues were confirmed by Western blot analysis. Western blot data also revealed that soluble endoglin, sFlt-1 and PlGF released by TCs were either in glycosylated or in complex formats.
Conclusions: TCs from PE placentas produced more endoglin, sFlt-1 and PlGF than those of normal TCs. Hypoxia could alter these soluble factor productions in TCs from PE. TCs released endoglin, sFlt-1 and PlGF were in complex formats.

282

Placental 11β-Hydroxysteroid Dehydrogenase 2 Activity in Preterm Pregnancy after Antenatal Betamethasone Treatment. Michael J Stark, Ian MR Wright, Vicki L Clifton.* *Mother and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia.*
AIM: Placental inactivation of cortisol by 11β-hydroxysteroid dehydrogenase-2 (11βHSD2) may protect the fetus from deleterious effects of both endogenous and exogenously derived glucocorticoid (GC). We have previously demonstrated a sexually dimorphic fetal response to inflammatory stressors in pregnancy. 11βHSD2 was implicated in the mechanisms contributing to the growth reduction observed. We aimed to investigate the relationship between placental 11βHSD2 activity, GC exposure, and birth weight in preterm neonates with respect to sex.
METHOD: Placental 11βHSD2 activity was measured by radiometric conversion assay in neonates at 24-28 weeks (n=20), 29-36 weeks (n=20), and 37-42 weeks (n=20) gestation. Mann Whitney U-test was used for comparison between groups and linear regression used to assess correlation between variables.
RESULTS: Total placental 11βHSD2 activity (activity rate x placental weight), but not placental 11βHSD2 activity rate (nmol/mg/hr), showed a significant correlation with gestational age (r=0.53, p<0.0001) and birth weight percentile (r=0.27, p<0.05). Small for gestational age (SGA) infants had lower placental 11βHSD2 activity (335.2 ± 47 *vs* 504.4 ± 51 nmol/mg/hr, p=0.02) and lower total placental 11βHSD2 activity (87.1 ± 14 vs 187.6 ± 29 μmol/hr, p=0.01) than infants of normal weight for gestational age. Preterm infants exposed to antenatal GC had a higher placental 11βHSD2 activity rate (479.8 ± 50.3 *vs* 299.6 ± 48.1 nmol/mg/hr, p=0.02), but not total placental activity (p=0.06), than those not exposed. A significant interaction between infant sex, steroid exposure, and total 11βHSD2 activity was evident for the preterm infants (F=4.98, p=0.03), with only females exhibiting a significant increase in activity following maternal GC exposure, reaching levels equal to those observed at term.
DISCUSSION: The sexually dimorphic response in 11βHSD2 activity following antenatal GC exposure has not previously been reported. The

definitive role of placental 11βHSD2 remains unknown. Placental metabolism of cortisol by 11βHSD2 may facilitate the development of an autonomous fetal hypothalamo-pituitary-adrenal axis. Following preterm birth adrenal insufficiency is associated with increased morbidity and mortality. The sexually dimorphic response exhibited by the female fetus may explain the increased incidence of poor outcome observed in males.

283

The Effect of IL-1β and IL-6 on OTR Protein Expression and Ligand Binding. Shirin Khanjani,[1] Yun S Lee,[1] Vasso Terzidou,[1] Mark R Johnson,[*1] Steven Thornton,[*2] Phillip R Bennett.[*1] *[1]Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, London, United Kingdom; [2]University of Warwick, Coventry, United Kingdom.*

We have previously reported that both IL-1β and IL-6 lead to increased expression of OTR at mRNA level peaking at 4 hours and reducing by 24 hours. We have also shown that IL1b leads to a transient increase in oxytocin binding to myocytes. In this study we have examined the effects of IL1β upon OTR protein expression and of IL-6 upon both protein expression and oxytocin binding.

Human myocytes in culture were incubated with IL-1β (1ng/ml) or IL-6 (NF-IL6, 10ng/ml). Cultures were stopped after 15, 30, 60 minutes, 2, 4 8, 12 and 24 hours and protein prepared for Western analysis. In parallel experiments OTR binding was measured in a competitive binding assay using the OT analogue ornothin vasotocin at 2, 4, 8 and 20 hours.

IL1β led to a modest 20% increase in OTR protein expression at between 2 to 8 hours. IL-6 also led to a modest 20% increase in OTR protein expression at between 30 minutes and to 4 hours decreasing to 80% between 8 and 24 hours. Binding was increased by IL-6 by 30% at 4 hours reducing to normal by 8 hours and to 80% of basal by 20 hours.

These data show that the biphasic effect of IL-β and IL-6 causing a transient increase in mRNA expression followed by a decrease, is also reflected at protein expression and receptor binding levels. The changes that we have seen in OTR expression are modest in comparison to changes seen in the expression of other labour-associated genes such as COX-2 or IL-8.

284

Fetal Sex Differences in Maternal Adrenal Steroid Levels at Three Time Points Prior to Preterm Delivery. Calvin J Hobel,[*] Chander P Arora, Meenu Sandhu, Racine Edwards-Silva. *Obstetrics & Gynecology, Cedars Sinai Medical Center, Burns and Allen Research Institute, Los Angeles, CA, USA.*

OBJECTIVE: The study was designed to assess the differences in the adrenal steroid levels between women with female and male fetus who delivered preterm.

HYPOTHESIS: Male fetus produces the precursors of estrogen and cortisol at a different rate than female fetus.

STUDY DESIGN: In a behavior in pregnancy study, 524 ethnically diverse women were prospectively followed at 18-20 weeks (T1), 28-30 weeks (T2) and 34-36weeks (T3). Maternal urine samples were collected at each stage for 17-a Hydroxy progesterone, Androstenedione and Cortisol and analyzed on the basis of fetal sex in fifty eight who delivered preterm (11.1%;19 female and 39 male).

RESULTS: Significant differences were found in the levels of 17-a- Hydroxy progesterone and Androstenedione in subjects with female and male fetus at all three time points. For 17-a-Hydroxyprogesterone (13.09 ng/ml vs 23.54 ng/ml at T1 and 27.79 ng/ml vs 39.24 ng/ml at T2, 42.36 ng/ml vs 47.46ng/ml at T3), p value <.001; For Androstenediaone (27.86 ng/ml vs 36.56 ng/ml at T1, 44.96 ng/ml vs 53.93 ng/ml at T2 and 60.82 ng/ml vs 66.63 ng/ml at T3), p-value < .001) with higher levels for both in male fetuses. Cortisol levels were significantly lower in the women with female fetus than those with male fetus only at T3; (45.4ng/ml vs 46.2ng/ml at T1; 56.63 vs 57.56 at T2 and 51.02 vs 69.4 ng/ml at T3, p-value <0.006). Average gestational age at delivery was 34.1 ± 2.6weeks for females and 34.3 ± 2.3 weeks for males.

CONCLUSION: This is the first report suggesting a distinct fetal sex dependant activation mechanism that determines maternal adrenal steroid levels during pregnancy prior to preterm birth. Fetal sex seems to play an important role in 17-a- Hydroxy progesterone production which can be converted to either androstenedione resulting in the synthesis of estrogens or to cortisol via 11-desoxycortisol. However, elevations in cortisol levels are similar in both male and females at T1 and T2 who deliver preterm except for the last time period (34-36 weeks) which may imply the increased risk of preterm birth for male fetuses.

285

Thrombin Inhibits Decidual Cell Progesterone Receptor Expression: Implications for Abruption Associated Prematurity. Charles J Lockwood,[*] Carlos Stocco, Edward Kuczynski, Graciela Krikun,[*] Rebeca Caze, Mizanur Rahman, Irina Buhimschi, Catalin Buhimschi, Frederick Schatz.[*] *Obstetrics/Gynecology & Repro. Sci, Yale University, New Haven, CT, USA.*

Objective: Placental abruption (decidual hemorrhage) is a leading cause of pre-term delivery (PTD). Thrombin generation during abruptions reflects enhanced access of circulating clotting factors to decidual cell-expressed tissue factor. The results of a multicenter trial established that progestin administration reduced the occurence of PTD in high-risk patients suggesting an integral role for progesterone receptor (PR) activity in protecting against PTD. Therefore, the current study evaluated the separate and interactive effects of thrombin and the synthetic progestin, medroxyprogesterone acetate (MPA) on PR mRNA levels and PR DNA-binding activity in term decidual cells (DCs).

Methods: Decidua from non-laboring patients (n=6) undergoing repeat Cesarean section was scraped from chorion and digested by collagenase-DNAase. Purified DCs were grown to confluence and passaged until flow cytometry indicated that the cultures were >99% free of CD45+-bearing cells. Confluent passaged term DC cultures were incubated with 10-8 M estradiol (E2) or E_2+ 10-7 M medroxyprogesterone acetate (MPA) for 7 days, then switched to serum-free defined medium with steroids ± thrombin. After 24h, DCs nuclear proteins and total RNA were extracted following standard methodology. Gel shift analyses were carried out using a radioactive labels consensus PR binding site. Quantification of total PR messenger was performed using real time RT-PCR and normalized to b-actin.

Results: In term DCs incubated with E_2, the mRNA level for PR was 29.2 ± 5.2 (mean ± SEM). The addition of MPA or thrombin decreased PR mRNA to 10.2 ± 2.8 and 10.5 ± 3.1, respectively. Additionally, the combination of E_2 +MPA + thrombin caused an even greater reduction in PR mRNA levels (3.0 ± 0.7). Moreover, gel shift analysis demonstrated that thrombin, added at 0.1 to 2.5 U/ml, elicited a concentration dependent decrease in PR DNA binding activity.

Conclusions: In view of the strong association between thrombin generation and abruption-related hemorrhage, the inhibitory effects elicited by thrombin on PR mRNA levels and PR DNA binding activity suggest that abruption leads to physiologic progestin withdrawal. This may account for the reduced risk of PTD when progesterone therapy is administered to high-risk patients.

286

Steroid Modulation of the Prostaglandin Receptors in Human Cervical Fibroblasts. Amy E Noll, Kimberly Hyatt, Dean A Myers.[*] *OB/GYN, University of Oklahoma HSC, Oklahoma City, OK, USA.*

BACKGROUND: Prostaglandins, key regulators of parturition, stimulate extensive biochemical changes in the composition of the cervical connective tissue. This cervical remodeling allows for cervical dilatation and in conjunction with increased uterine contraction results in subsequent birth of the fetus. Prostaglandin E2 (PGE2) and F2α are the major PGs mediating cervical ripening. PGE2 signals through four major G-protein coupled receptors (EP1-EP4). Detailed studies examining regulation of EP1-EP4 expression need to be conducted. Estrogen (E2) and progesterone (P4) are also known to play a major role in cervical ripening. To date, few studies have addressed regulation of EP receptor expression in cervical fibroblasts, a major cell type involved in tissue remodeling. The purpose of the following study was to 1) determine if human cervical fibroblasts (HCFs) express either progesterone or estrogen receptors (PR and ER respectively) and 2) to examine the effect of estradiol and progesterone on EP and FP receptor expression.

MATERIALS AND METHODS: Human cervical fibroblasts previously established from explants of cervical tissue (non-pregnant) were maintained in vitro in DMEM supplemented with 10% FBS. Total RNA was prepared from HCFs and subjected to reverse transcription PCR (RT-PCR) using primers specific for ERα, ERβ, PR total (PR-A+B) or PR-B. HCFs were treated with E2 or P4 (100, 10, 1.0, 0.1, 0.01 nM) for 24hrs. Total RNA was prepared and subjected to semi-quantitative qRT-PCR for EP1, EP2, EP3, EP4 and the prostaglandin F (FP) receptor.

RESULTS: Based on RT-PCR, HCFs were observed to express PR-A and PR-B; in addition HCFs express ERα but not ERβ. Based on RT-PCR, HCFs express EP1, EP2, EP3, EP4 and FP; the relative abundance of mRNA, based on Ct values was EP4>EP1>EP3=FP>EP2. E2 decreased EP2 mRNA in a dose dependent manner to 55% of control; maximal suppression was observed at 1nM. P4 similarly decreased EP2 mRNA to 47% of control; maximal

FRIDAY

suppression was observed at 100nM. E2 increased EP4 mRNA by 44% with maximal stimulation noted at 10nM. P4 had no effect on EP4 mRNA. Neither E2 nor P4 had an effect on EP1, EP3 or FP mRNA.
CONCLUSIONS: HCFs represent direct targets for estrogen and progesterone in regulating HCF function. Based on our findings these steroids potentially regulate the responsiveness of HCFs to prostaglandins by selectively modifying EP receptor expression.

287

Prostaglandin Receptors Expression in Cultured Uterine Smooth Muscle Cells Exposed to Inflammatory Inducers. Aimin Li,[1] Juan C Felix,[1,2] Richard H Lee,[1] John K Jain,[*1] Thomas M Goodwin.[*1] *[1]Dept. of Ob/Gyn, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; [2]Dept. of Pathology, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA.*
Objectives:
Previous studies have indicated that spontaneous preterm labor is a host inflammatory response disease, and that prostaglandin E2 and F2a, mediated through their respective receptors named EP1-4 and FP are believed to play important roles in the myometrial contraction and the initiation of labor. The present study was undertaken to determine the effect of lipopolysaccharide (LPS) on inflammatory cytokines and prostaglandin pathway gene expression patterns using human uterine smooth muscle cell (USMC) line as a model.
Methods:
USMC (Cambrex Bio Science Walkersville, Inc), passages 4-10 were exposed to 0, 0.1, 1 and 10 µg/ml of LPS for 6, 24 and 48 hours. The production of IL-8, IL-6 and TNFalpha was determined using enzyme-linked immunosorbent assay (ELISA) and the expression of prostaglandin pathway genes, specifically, cyclooxygenase (COX)-2, EP1-4, FP, and oxytocin receptor (OXTR) was determined by real-time PCR. Immunofluorescent staining for FP was also performed.
Results:
LPS at concentration of 1 and 10µg/ml increased the production of IL-8, IL-6 and TNFalpha and the expression of COX-2, EP3, FP and OXTR mRNA while decreasing EP2 mRNA after 24 and 48 hours. Immunofluorescent staining revealed that FP receptor appeared to be located in the nucleus as well as evenly dispersed throughout the cytosol of uterine smooth muscle cells and the nuclear staining intensified after LPS treatment.
Conclusions:
USMC responded to LPS with increased secretion of IL-6, IL-8 and TNF alpha and expression of contractile-associated genes COX-2, EP3, FP and OXTR. Based on these results, we propose that IL-6, IL-8 and TNF alpha are involved in the initiation and promotion of labor under inflammatory conditions by inducing PGE2 or PGF2a production, EP3, FP and OXTR expression in myometrium.

288

Interleukin-1 Induced Cyclooxygenase-2 and Prostaglandin Expression in Term Decidual Cells Are Suppressed by Glucocorticoids and Progestin. Hakan Cakmak,[1] Lynn F Buchwalder,[1] Carlo Saccardi,[2] Aydin Arici,[*1] Frederick Schatz,[*1] Charles J Lockwood.[*1] *[1]Obstetrics/Gynecology & Repro. Sci, Yale University, New Haven, CT, USA; [2]Gynecological Science and Human Reproduction, University of Padua School of Medicine, Padua, Italy.*
Objective: The induction of cyclooxygenase-2 (COX-2) and increased synthesis of prostaglandins (PGs), PGE_2 and $PGF_{2\alpha}$, by inflammatory cytokines play a central role in the initiation pre-term delivery (PTD). We recently found that medroxyprogesterone acetate (MPA) blunts this increase in COX-2 expression and PG synthesis. MPA is known to possess both glucocorticoid and progestin properties. Thus to elucidate whether this effect was due to glucocorticoid or progestin actions, we examined the effects of MPA, Dexamethasone (Dex), and a pure progestin (PP) on interleukin-1 beta (IL-1) induced COX-2 expression and PG production in cultured term decidual cells (DCs).
Materials & Methods: DCs were isolated, purified and grown to confluence. After priming with 10(-8) M estradiol (E2) or E2 plus 10(-7) M of either MPA or Dexamethasone (Dex) synthetic pure progestin (PP; ORG 2058) for 7 days, cultures were incubated in a defined medium with corresponding steroid(s) with or without IL-1 (1 ng/ml). After 24 hr, COX-1 and COX-2 protein levels in cell lysates, and PGE_2 and $PGF_{2\alpha}$ levels in culture media were measured by Western blotting and ELISA, respectively. COX-1 and COX-2 mRNA levels were assessed by quantitative real-time RT-PCR after 6hr.
Results: Neither COX-1 protein nor mRNA expression changed across the different treatment groups. COX-2 protein and mRNA expression was upregulated by IL-1b, but this regulation was blunted by MPA and Dex, and PP to a lesser degree. The addition of IL-1 increased secreted PGE_2 and $PGF_{2\alpha}$ levels by 206±123-fold and 27±7-fold in cultures maintained in E_2, respectively ($p<0.05$; mean±SEM, n=4). The addition of MPA and Dex both significantly reduced the effect of IL-1 on PGE_2 and $PGF_{2\alpha}$ secretion by more than 50%, while PP reduced this effect by only 25%.
Conclusions: IL-1 increased COX-2 and PG production in term DCs, while MPA and Dex, and progestin, less so, blunted these effects. Given the importance of PGs in the pathogenesis of PTD, their inhibition by both glucocorticoids and progestin suggests that exogenous glucocorticoids, in addition to progesterone, may be a better strategy in the the prevention of PTD.

289

Molecular Markers of Labour in Human Myometrium. Mark Tattersall,[1] Suren R Sooranna,[1] Peta Grigsby,[2] Leslie Myatt,[*2] Phillip R Bennett,[*1] Mark R Johnson.[*1] *[1]Obstetrics & Gynaecology, Imperial College Parturition Research Group, London, United Kingdom; [2]Obstetrics & Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*
Introduction: Although the myometrial contractions of labour signal the normal physiological end-point of pregnancy the biochemical onset of labour may occur at or before term via a series of changes in expression of labour-related genes. Historically, expression of oxytocin receptor (OTR) and connexin-43 (Cx-43) were suggested as 'marker genes' of the terminal myometrial phenotype. However, the major role played by prostaglandins and inflammatory cytokines in labour implies that expression of prostaglandin H synthase (PGHS-2), Il-8 and Il-1β may also be suitable candidates to define myometrium as being in labour. Our aim was to explore which of these genes were definitive markers for preterm and term labour.
Methods: Paired samples of upper and lower segment myometrium were taken from pregnant women undergoing LSCS either before or after the onset of term or pre-term labour and frozen. RNA was extracted and converted to cDNA (n=9 for PTNL; PTL; n=8 for TNL; n=10 for TL). Copy numbers of PGHS-2, OTR, Cx-43, Il-8 and Il-1β and beta-actin were measured by qPCR using a Rotor-Gene™. Western blotting was performed for PGHS-2, OTR, Cx-43 and beta-actin. Immunocytochemistry for PGHS-2 and OTR was performed on myometrial tissue sections.
Results: PGHS-2 expression increased in both the upper ($p<0.01$) and lower ($p<0.003$) segment myometrium in term labour. A similar pattern was seen with Il-1β expression ($p<0.01$ and $p<0.006$ respectively). However, Il-8 expression was significantly increased in the lower myometrial segment in preterm ($p<0.047$) and term labour ($p<0.006$). No effect of labour was seen in either OTR or Cx-43 expression. Immunoblotting for PGHS-2 was strongest in the term labour samples, whereas OTR showed no differences in the upper myometrium and a tendency to decrease with labour in the lower myometrium. No differences were seen for Cx-43. Immunocytochemical staining was strongest in the term labour samples for PGHS-2. Individual cells within tissue sections showed strong staining for OTR in upper and lower segment myometrium under all conditions.
Conclusions: These data show that PGHS-2, Il-1β and Il-8 may be more appropriate 'marker genes' of the terminal myometrial phenotype. This study also highlights differences between term and preterm labour and upper and lower segments of myometrium.

290

Nuclear Progesterone Receptor Expression and Function in the Human Pregnancy Myometrium. Amy Merlino,[3] Vernon Cannon,[2] Toni Welsh,[1] Brian Mercer,[*3] Juan Li Yi,[1] Huiqing Tan,[1] Sam Mesiano.[*1] *[1]Reproductive Biology, University Hospitals Case Medical Center, Cleveland, OH, USA; [2]Department of Obstetrics and Gynecology, Northwestern University, Evanston, IL, USA; [3]Reproductive Biology, Case-MetroHealth Medical Center, Cleveland, OH, USA.*
OBJECTIVE: In most animals labor is initiated by progesterone withdrawal. The mechanism for progesterone withdrawal in human parturition is unclear since labor occurs without a decrease in circulating progesterone levels. It is hypothesized that human parturition involves a functional progesterone withdrawal whereby changes in myometrial progesterone receptor (PR) expression decrease progesterone responsiveness. Our previous mRNA data suggest that functional progesterone withdrawal is caused by increased expression of PR-A (a suppressor of progesterone actions) relative to PR-B (the principal mediator of progesterone actions) i.e., the PR-A/PR-B expression ratio. The objective of this study was to test the PR-A/PR-B hypothesis at the protein and functional levels.

STUDY DESIGN: Lower uterine segment biopsies were obtained at the time of c-section from consenting women at preterm and not in labor (PTNIL; n=6), at term and not in labor (TNIL; n=6) and at term and in labor (TIL; n=6). Western blotting for nPRs was performed on total cell lysates using the PgR1294 PR antibody. Cellular localization of PRs was determined by PgR1294-immunohistochemistry. Relative abundances of PR-B and total PR mRNAs were measured by quantitative RT-PCR and PR-A/PR-B function was assessed by co-transfecting PHM1-31 cells with PR-A and PR-B and measuring activity of a progesterone-responsive luciferase reporter construct.
RESULTS: The myometrial PR-A/PR-B protein ratio increased significantly with advancing gestation (0.5 before 32 weeks vs 1.0 at term, $p < 0.001$) and with the onset of labor at term (1.0 not in labor vs 2.5 in labor, $p < 0.01$). The increase was due to increased abundance of PR-A; PR-B levels remained relatively constant. PRs were localized exclusively to the nuclei of myometrial cells. PR mRNA levels were consistent with the changes in PR proteins. In PHM1-31 cells PR-A repressed the transcriptional activity of PR-B at a PR-A/PR-B ratio similar to that of term laboring myometrium.
CONCLUSION: These data support the hypothesis that functional progesterone withdrawal in human parturition is mediated by an increase in expression of PR-A relative to PR-B.

291

The Mechanism of Upregulation of Oxytocin Receptor (OTR) by NFκB and C/EBP. Shirin Khanjani,[1] Yun S Lee,[1] Vasso Terzidou,[1] Mark R Johnson,[*1] Steven Thornton,[*2] Phillip R Bennett.[*1] *[1]Imperial College Parturition Research Group, London, United Kingdom; [2]University of Warwick, Coventry, United Kingdom.*
We have shown, in transient transfections in human myocytes, that the combination of transcription factors NFκB and C/EBP leads to a synergistic increase in OTR promoter activity greater than either alone. This effect is independent of any NFκB binding site but is mediated through the distal 405bp region of the promoter. We have undertaken studies to refine the region which responds to NFκB or C/EBP in combination.
Deletions were made in a 1.1kb OTR promoter construct. This construct was transfected into myocytes together with NFκB and/or C/EBP expression vectors. A series of deletions in the OTR promoter were made and their effect upon synergy examined. These studies showed that the critical region lies in 20bp -710 to -691. Two C/EBP binding sites had sequences within this region. Deletions across these sites eliminated synergy.
To date the synergistic effect of NFκB and C/EBP upon OTR has been shown only at promoter level. We therefore performed immunoblots to determine the time of peak expression of overexpressed proteins. Both NFκB and C/EBP were endogenously expressed. Overexpression peaked at 24 to 36h. TaqMan RT-PCR showed that overexpression of NFκB or C/EBP caused a two fold increase at 24h whilst NFκB and C/EBP combined caused a 20 fold increase in OTR mRNA. However western analysis showed that NFκB or C/EBP alone had no effect upon protein expression whilst their combination caused a 10% increase at 36h.
We conclude that the synergistic effect of NFκB and C/EBP upon OTR promoter activity is via C/EBP sites between -710 to -691. This effect upon OTR promoter activity is dramatic, but the effect upon endogenous mRNA synthesis and protein expression is less marked. We have found in other studies that cytokines stimulate significant changes in OTR mRNA but small changes in protein concentrations and ligand binding (Terzidou et al 2006, Khanjani et al SGI 2007) and that there are no changes in OTR mRNA and protein levels in myometrium with labour (Tattershall et al SGI 2007). This suggests processes which stimulate OTR expression are likely to have small effects upon protein concentrations.

292

Does Ethnicity Predict Response to Tocolysis? Tracy A Manuck, Anibal Martinez-Borges, Heather L Mertz. (SPON: David C Merrill). *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*
OBJECTIVE: Non-Caucasian women experience preterm labor (PTL) and delivery more frequently than women of other ethnicities. This has been partially attributed to genetic differences. Interactions between genes and pharmocotherapy have also been reported. This study was conducted to identify differences in tocolytic response among African American, Caucasian, and Hispanic women.
STUDY DESIGN: A retrospective review of 255 consecutive patients, managed by a single university practice, at 22-34 weeks gestation with PTL and intact membranes from January 2003-June 2006 was performed. Patients received magnesium, Indocin, or both. Women were grouped by ethnicity and compared. Variables examined included maternal age, parity, cervical dilation at presentation, tocolytics administered, presence of bleeding, prior preterm delivery, prior cervical surgery, tobacco abuse, gestational age at presentation and at delivery, APGAR scores, infant birthweight, and presence of a twin gestation. ANOVA and Chi-square analysis were used where appropriate. Stepwise multiple logistic regression was also performed. Response to tocolysis, defined as prolongation of pregnancy for at least 48 hours, was the outcome variable.
RESULTS: The analysis included 238 patients; 35.3% African American, 50.8% Caucasian, and 13.9% Hispanic. All variables analyzed were similar between ethnic groups. In 29 patients, delivery was imminent and tocolytics were not administered. 28.7% of Caucasian, 33.8% of African American, and 47.4% of Hispanic patients did not respond to tocolysis. In the multivariate model, a trend toward Hispanic ethnicity as an independent risk factor for non-response to tocolysis was noted as compared to Caucasians, although this did not reach statistical significance (OR 2.24, 95% CI 0.82-6.06).
CONCLUSION: This study is limited by a small sample size, but suggests ethnicity may influence response to tocolysis. Further studies are needed to explore the effect of ethnicity on genetic expression, and elucidate interactions between the process of PTL, tocolytics, and genetics.

293

Unique Global Patterns of Placental Gene Expression Promote Preterm Delivery (PTD) in Preeclampsia (PE), Intrauterine Growth Restriction (IUGR), and Chorioamnionitis (CA). Seth Guller,[*] Catalin S Buhimschi, Se-Te J Huang, Yuehong Ma, Edward Kuczynski, Charles J Lockwood,[*] Irina A Buhimschi. *OB/GYN & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
OBJECTIVE: Compelling evidence suggests that PE, idiopathic IUGR, and labor-associated PTD manifest common but also distinct pathways of placental dysfunction. We hypothesize that unique global patterns of placental gene expression are responsible for the widely divergent clinical manifestations of PE, IUGR, and CA.
METHODS: Placental gene expression profiles (n=3) were generated from women, whose pregnancies were complicated by severe PE [gestational age (GA): 29.7 ± 1.0 weeks], idiopathic severe IUGR (GA, 33.3 ± 0.5 weeks), PE+IUGR (GA, 27.9 ± 2.2 weeks), CA (GA, 27.8 ± 2.2 weeks), idiopathic preterm delivery, IPTD, (GA, 30.0 ± 2.7 weeks), and those at term (39.1 ± 0.3 weeks). Preterm groups were matched for GA. Total RNA was isolated from placental specimens and was hybridized to an Affymetrx HG_U133 Plus 2.0 chip containing approximately 47,400 human genes and expressed sequence tags (ESTs). The microarray results were analyzed with GCOS 1.4 and GeneSpring GX 7.3.1 software. Following per chip and per gene normalization and exclusion of absent genes, comparisons among groups were conducted based on only those genes identified to be regulated vs IPTD and term groups by one-way ANOVA using a 2-fold cut-off ($p < 0.05$).
RESULTS: The ratios of up-and down-regulated genes in a specific group/genes regulated in common were unique and characteristic for PE, IUGR, PE+IUGR, and CA groups (Table 1). The proportion of genes regulated in common in the 4 groups was ≤ 40%, and for several comparisons was only 1-2%.
Conclusions: PE, IUGR, and CA are characterized by distinct placental gene expression profiles for both up- and down-regulated genes. This indicates that a unique set of placental genomic responses may be responsible for the diverse panel of clinical manifestations.

Table 1. Placental Gene Expression Profiles

Group Comparison	Up-Regulated	Down-Regulated
PE vs IUGR	181/2	59/2
PE vs CA	45/0	60/1
PE vs PE+IUGR	140/43	48/13
IUGR vs PE	5/2	18/2
IUGR vs PE+IUGR	6/1	18/2
CA vs PE	21/0	16/0
PE+IUGR vs PE	63/43	152/13
PE+IUGR vs IUGR	105/1	163/2

FRIDAY

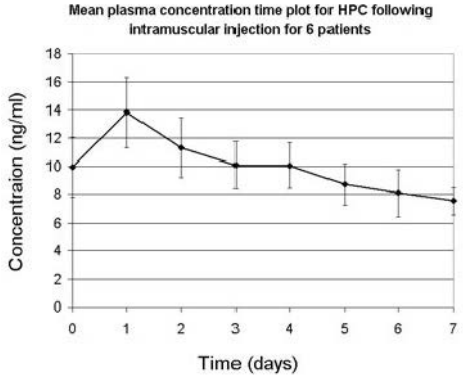
294

Pharmacokinetics of 17-Alpha-Hydroxyprogesterone Caproate (17-OHPC) in Women with Twin Gestation. Steve N Caritis,* Raman Venkataramanan. *for the Network of Maternal – Fetal Medicine Units, Bethesda, MD, USA.*

Objective: Despite the increasing usage of 17-OHPC for prevention of preterm birth in high risk groups, no data exist as to the pharmacokinetics of this treatment in pregnant women. The objective of this study was to determine the pharmacokinetics of 17-OHPC in women with twin gestation participating in a multicenter RCT of 17-OHPC vs placebo in the prevention of preterm birth.

Methods: We recruited 6 women who were receiving weekly IM injections of 17-OHPC in castor oil from the time of recruitment (16 0/7- 20 6/7 weeks) until 35 weeks gestation. The PK study was performed during the second or third trimester after at least 4 injections had been administered. For each PK study, plasma was collected prior to the next injection and daily for seven consecutive days after an injection. Plasma 17-OHPC concentrations were quantified using LC-MS with a sensitivity of 0.5ng/ml and a coefficient of variation of less than 10%.

Results: The mean (± SE) plasma concentrations prior to and after IM injection of 250 mg 17-OHPC are depicted in the figure below.



The PK parameters (mean ± SE) for these 6 women include:

AUC(ng/ml/day)	Cmax (ng/ml)	Tmax (days)	T 1/2(days)
70.2 ± 29.3	13.0 ± 6.2	1.2 ± 0.45	9.1 ± 4.2

AUC is area under the plasma concentration time curve.

Cmax is maximum concentration

Tmax is time of maximal concentration.

T 1/2 is half life

Conclusions: These are the first data reporting plasma concentrations and pharmacokinetic parameters with 17-OHPC therapy in pregnant women. AUCs differ three fold suggesting substantial individual variation in release and metabolism. It is unclear if this variation may lead to a differential response in efficacy. The long half life suggests that release from the castor oil depot rather than metabolism of 17-OHPC dictates the plasma concentration – time profile. Whether these concentrations differ substantially from those seen in singleton gestations remains to be determined.

295

Progestin and Thrombin Enhance Tissue Factor Expression in Term Decidual Cells: Implications for Protection Against Abruption-Related Preterm Birth. Frederick Schatz,*[1] Felice Arcuri,[2] Paolo Toti,[2] Lynn F Buchwalder,[1] Rebeca Caze,[1] Mizanur Rahman,[1] Graciela Krikun,*[1] Joseph ST Huang,[1] Charles J Lockwood.*[1] *[1]Obstetrics/Gynecology & Repro. Sci, Yale University, New Haven, CT, USA; [2]Human Pathology &Oncology, University Siena, Siena, Italy.*

Objective: The hypercoaguable state of pregnancy protects against the hemostatic challenges of delivery. Tissue factor (TF) is the primary initiator of hemostasis via thrombin generation. In human endometrium, TF expression is enhanced during progestin-induced decidualization of stromal cells in the luteal phase and in decidual cells (DCs) of first trimester placentas. The current study determined whether TF expression also includes DCs of term placentas and is regulated when term DCs are incubated with medroxyprogesterone acetate (MPA) and thrombin added separately or together.

Study Design: Immunohistochemical (IHC) TF expression was observed in specimens of normal term decidua (n=4). Purified DCs from normal term deliveries were passaged until >99% free of CD45+ cells by FACS. Confluent DCs were primed with 10^{-8} M estradiol (E_2) or $E_2 + 10^{-7}$ M medroxyprogesterone acetate (MPA) for 7 days, then incubated with corresponding steroids +/- thrombin. After 24h, DCs proteins were assayed for TF by ELISA. Total RNA extracted from parallel cultures were used to assess TF mRNA levels by quantitative real-time RT-PCR.

Results: IHC observations localized TF at the cell membranes of DCs in term placentas. In term DC monolayers (mean ± SEM, n=10) membrane-associated TF levels incubated were 248 ± 69 with E_2 and 634 ± 171 pg/ml/ug total cell protein with E2 + MPA. Compared with E2, thrombin (2.5 U/ml) enhanced TF output in incubations with E_2 (2.8 ± 0.3-fold, $p< 0.05$) and further with E_2 + MPA (11.5 ± 6.6-fold, $p<0.05$). TF mRNA levels corresponded to changes in ELISA TF protein levels.

Conclusions: Under sustained progestin exposure term DCs continue to express TF. Localized at the cell membrane, DC-expressed TF binds to circulating clotting factors. The latter convert pro-thrombin to thrombin, which promotes hemostasis via fibrin formation. Since thrombin is generated during abruptions (decidual hemorrhage) further enhancement of TF expression by the DCs observed *in vitro* suggests a feed forward mechanism that confers protection against abruption-related pre-term delivery.

296

Proteomic Profiling Detects Calgranulin B in Cervicovaginal Fluid from Women Who Deliver Preterm. Rita S Leite, Amy G Brown, Samuel Parry.* *Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: To identify candidate protein biomarkers in cervicovaginal fluid of pregnant women that may be linked to preterm labor and delivery.

Methods: Cervicovaginal fluid was collected from women who presented to our obstetrical triage unit with uterine contractions between 20 and 32 weeks gestation. Women were excluded if their cervix was greater than 2 cm dilated, vaginal bleeding was present, or the fetal membranes were ruptured. Obstetrical outcomes were determined by chart review. Samples were flash-frozen, stored at minus 80 C, spotted onto WCX2 chips, and analyzed using SELDI-TOF-MS. Spectra were obtained for each sample and peak differences were determined using Biomarker Wizard Software. Protein peaks with significantly greater intensity in women who delivered preterm (less than 37 weeks) were identified using a Q-STAR XL tandem mass spectrometer (ABI) equipped with a PCI-1000 ProteinChip Interface (Ciphergen).

Results: Demographic characteristics were similar between women who delivered preterm (n=19) and women who delivered at term (n=46). Proteomic analysis revealed 9 peaks that were significantly different between preterm and term deliveries, of which 4 protein peaks had significantly greater intensity (P values less than 0.002) in women who delivered preterm. Two of these peaks were determined to be fragments of calgranulin B: candidate markers at 2,178 Da (amino acid sequence: ASHEKMHEGDEGPGHHHKPG) and 2,509 Da (amino acid sequence: RKDLQNFLKKENKNEKVIEH).

Conclusions: We conclude that: 1) there is a distinctive cervicovaginal protein signature differentiating women with preterm contractions who deliver preterm and women with preterm contractions who eventually deliver at term; and 2) specific calgranulin B fragments found in higher intensity in samples from women who delivered preterm may play a role in inflammation-induced preterm delivery. Calgranulin A and B form a heterodimer known as calprotectin, which is stored in the cytoplasm of neutrophils and macrophages and participates in the host defense response against pathogenic microbes in the female genital tract. Calgranulin B previously has been detected in amniotic fluid and serum of pregnant women with preterm labor and intra-amniotic infection. Detection of markers such as calgranulin B might have application in the identification of women at highest risk of spontaneous preterm delivery.

297

Identification of Single Nucleotide Polymorphisms in Maternal Genes Associated with Spontaneous Preterm Birth. Errol Norwitz,*[1] Thomas Morgan,[2] Victoria Snegovskikh,[1] Edward Kuczynski,[1] Hee Joong Lee,[1] Frederick Schatz,*[1] Se-Te Joseph Huang,[1] Catalin Buhimschi,[1] Edmund Funai,*[1] Irina Buhimschi,[1] Guoyang Luo,[1] Sonya Abdel-Razeq,[1] Charles Lockwood.*[1] *[1]Ob/Gyn, Yale University, New Haven, CT; [2]Pediatrics, Washington University, St. Louis, MO.*

OBJECTIVE: Familial clustering, racial disparities, and the high incidence of recurrent preterm birth (PTB) suggest a critical role for maternal genetic factors in the timing of labor. This study investigates the association between 128 single nucleotide polymorphisms (SNPs) in 87 candidate maternal genes and spontaneous PTB.

METHODS: Consecutive patients with spontaneous PTB were identified from the March of Dimes PERI project (#20-FY03-30) at NYU, NY and Yale, CT from Jan 1989-June 2005. Cases were matched (4:1) with uncomplicated term deliveries (controls). DNA was extracted from stored buffy coats and genotyping performed using established primers. All specimens were linked with demographic and medical data abstrated from maternal/neonatal records. Genotyping was performed using Sequenom MALDI-TOF platform with individualized assay designs created by automated Spectrodesign software (Sequenom, San Diego, CA). Data were analyzed by permutation-based exact chi-square test for 2x3 genotype tables and by logistic regression analysis with adjustment for race (SPSS, Chicago, IL). $p<0.05$ identified a significant association. The total number of positive associations was placed in stochastic context by computer simulation (Resampling Stats, Inc). The analysis was confined to Hispanics due to lack of power for other ethnic groups.
RESULTS: Genotyping was successful in over 96% of samples. Hardy-Weinberg expectations were met in all but 3 SNPs. In Hispanics, 4 gene variants were positively association with PTB: ENPP1S121 ($p=0.003$); CCR5S1 ($p=0.007$); CYP2C9S144 ($p=0.047$); IL6S174 ($p=0.045$). At $p<0.05$, 4 positive genes would be expected by chance in 85% of the time based on 10,000 computer simulations involving 120 genetic comparisons; however, 2 positive genes at $p<0.01$ would occur in only 12% of computer simulations. The rare allele of ENPP1 SNP was significantly overrepresented in PTB cases [OR 2.1 (95% CI: 1.4, 3.2)].
CONCLUSION: SNPs in 4 candidate genes appear to be associated with spontaneous PTB. Although not more than expected, the strong association of the ENPP1 SNP was particularly compelling. Additional studies are ongoing to further investigate these associations.
MOD #21-FY05-1250 (to EN).

298

Single Nucleotide Polymorphisms in the Surfactant Protein-A (SP-A) Gene and Spontaneous Preterm Birth. Errol Norwitz,[*1] Thomas Morgan,[2] Victoria Snegovskikh,[1] Vineet Bhandari,[3] Edward Kuczynski,[1] Hee Joong Lee,[1] Frederick Schatz,[*1] Se-Te Joseph Huang,[1] Catalin Buhimschi,[1] Edmund Funai,[*1] Irina Buhimschi,[1] Victor Rosenberg,[1] Charles Lockwood.[*1] *[1]Ob/Gyn, Yale University, New Haven, CT; [2]Pediatrics, Washington University, St. Louis, MO; [3]Pediatrics, Yale University, New Haven, CT.*
OBJECTIVE: Recent studies have suggested that SP-A may be critical to the onset of labor both in mice (PNAS 2004;101:4978) and humans (SGI abstract #665, 2006). Single nucleotide polymorphisms (SNPs) in the human SP-A gene have been associated with respiratory diseases, including neonatal respiratory distress syndrome, bronchopulmonary dysplasia, and adult respiratory distress syndrome. Their association with preterm birth has not been previously examined. This study investigates the association between the +62(G>A) SNP in the maternal SP-A gene and preterm birth.
METHODS: Consecutive patients with spontaneous (non-iatrogenic) preterm birth were identified from the March of Dimes Perinatal Emphasis Research Initiative project (MOD # 20-FY03-30) at New York University, NY and Yale University, CT from Jan 1989-June 2005. Cases were matched (4:1) with uncomplicated term deliveries (controls). DNA was extracted from stored buffy coats and genotyping performed using established primers. All specimens were linked with demographic and medical data abstrated from maternal/neonatal records. Genotyping was performed using the Sequenom MALDI-TOF platform with individualized assay designs created by automated Spectrodesign software (Sequenom, San Diego, CA). Data were analyzed by permutation-based exact chi-square test for 2x3 genotype tables and by logistic regression analysis with adjustment for race (SPSS, Chicago, IL).
RESULTS: Genotyping was successful in 98.7% of samples. Hardy-Weinberg chi-square genotype distributions were as expected in cases ($p=0.99$) and controls ($p=0.24$). The overall genotype distributions of the +62(G>A) variant in cases (n=89) was GG=51, GA=33, AA=5; and GG=242, GA=157, AA=38 in controls (n=437). In logistic regression analysis with adjustment for race, there was no association between the +62(G>A) genotype and preterm birth ($p=0.619$).
CONCLUSION: The +62(G>A) SNP in the human SP-A gene is not associated with spontaneous preterm birth. Further studies are required to better define the role of SP-A in the onset of labor, both at term and preterm.
Funded by March of Dimes #21-FY05-1250 (to EN).

299

Mitogen-Activated Protein Kinase (MAPK) and MAPK Phosphatase (MPK) Expression in Human Myometrium and Fetal Membranes. Suren R Sooranna,[1] Neelam Engineer,[1] Peta Grigsby,[2] Phillip R Bennett,[*1] Leslie Myatt,[*2] Mark R Johnson.[*1] *[1]Obstetrics & Gynaecology, Imperial College Parturition Research Group, London, United Kingdom; [2]Obstetrics & Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*
Introduction: MAPKs are evolutionary conserved serine-threonine signalling kinases that connect receptors at the cell surface to intracellular regulatory targets. They are central to mediating gene regulation in intrauterine tissues during parturition. There are 3 distinct groups of MAPKs: 5 extracellular signal-related kinases (MAPK1, 3, 4, 6 and 7), 3 jun amino-terminal kinases (MAPK8, 9 and 10), and 4 p38 kinases (MAPK11, 12, 13 and 14). The MAPKs are activated by phosphorylation through a cascade of other kinases and are inactivated by 3 common MAPK phosphatases (MKP-1, 2 and 3). The importance of MAPKs phosphorylation in myometrium and fetal membranes at the time of parturition led us to explore whether regulation of MAPK and MAPK phosphatase genes also occurred in preterm and term labour.
Methods: Paired upper and lower segment samples of myometrium from pregnant women undergoing LSCS either before or after the onset of term (TNL and TL) or pre-term (PTNL and PTL) labour were taken and frozen for extraction of RNA. Matching samples of amnion and chorion from the same patients were similarly frozen (n=6 in all cases). RNA was extracted and converted to cDNA. Copy numbers of MAPK1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14 and MKP-1, 2, 3 and beta-actin were measured by quantitative real-time PCR using a Rotor-Gene™ (Corbett Research, Australia).
Results: All 12 MAPK and 3 MKP genes were expressed in both upper and lower segment myometrium, and in amnion and chorion of preterm and term labouring and non-labouring women. There was remarkably little change in expression between non-labour and labour states in myometrium, amnion and chorion for these 15 genes. MAPK3 (ERK1) and 14 (p38alpha) were the most abundant MAPKs in the amnion and the chorion and were significantly greater than in the myometrium ($p<0.05$ in all cases). In addition to these 2 MAPKs, the JNKs were prominent in the myometrium. Of the phosphatases, MKP-1 showed the highest expression in all tissues examined.
Conclusions: These data show that all the MAPK and the 3 MKP genes were expressed in myometrium, amnion and chorion. As there is no difference in expression of the proteins regulation of their activity at parturition is apparently primarily by phosphorylation.

300

Analysis of Expression of the mk44 Variant in Human Pregnant Myometrium. Audrey T Moynihan,[1,2] John J Morrison,[*1] Terry J Smith.[2] *[1]Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Galway, Ireland; [2]National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland.*
OBJECTIVE: The BK_{Ca} channel α-subunit has revealed many different splice variants. One such variant, a 132 bp exon encodes an additional 44 amino acid segment, which has decreased sensitivity to calcium. It is expressed in the uterus, and has been shown at the mRNA level to increase as a proportion of the total channel α-subunit in human myometrium with the onset of labour. This suggests a contribution during the labour process. The goal of this study was to determine the functional significance of the 44 aa spliced exon of the BK_{Ca} alpha subunit (mK44) in non-pregnant (NP), pregnant non-labouring (PNL) and pregnant labouring (PL) myometrium samples.
METHODS: Protein was extracted from human NP, PNL, and PL myometrium and quantified. Myometrial protein samples (35 µg) were resolved on a 7.5% SDS-PAGE gel, blotted and probed with rabbit anti-132 antibody (1:500), an antibody generated specifically to the 132 spliced exon of the BK_{Ca} α-subunit. Protein expression levels of the BK_{Ca} alpha subunit containing the 132 bp spliced exon were determined by laser densitometric analysis. Differences between the groups were determined by analysis of variance (ANOVA) followed by post-hoc Tukey analysis where appropriate, using the statistics program SPSS. A P value of < 0.05 was considered to be statistically significant.
RESULTS: Western blot analysis of the BK_{Ca} channel α-subunit mK44 variant in NP, PNL, and PL myometrium using an anti-132 antibody revealed no significant differences in the expression of the variant between NP, PNL, or PL groups following ANOVA and post-hoc testing ($P<0.05$).

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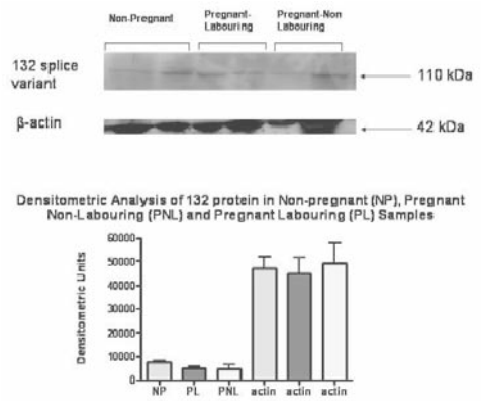


CONCLUSIONS: This study has provided insight into the functional significance of the BK_{Ca} specifically the 44 aa spliced exon-containing variant of the channel. As the expression of the whole channel decreases at labour, the channels expressing the mK44 variant remain the same. Given that this variant has decreased sensitivity to calcium, this may facilitate uterine contractility at labour.

301

Lactobacilli Supernatant Inhibits TNF-α Production and COX_2 Expression in LPS-Activated Placental Trophoblasts. Maryam Yeganegi,[1,3] Carole Watson,[3] Sung Kim,[2] Gregor Reid,[2] John Challis,[*1] Alan Bocking.[*1,3] *[1]Dept. of Physiology & Ob/Gyn, Univ. of Toronto, Toronto, Canada; [2]Dept. of Microb. & Immun, Univ. of Western Ontario, London, Canada; [3]Samuel Lunenfeld Research Institute, Mount Sinai Hosp, Toronto, Canada.*

Objective: Bacterial Vaginosis(BV) is characterized by the presence of gram-negative bacteria, and the absence of endogenous *Lactobacillus*. BV is associated with a 1.4-fold increased risk of preterm birth(PTB). Pathogenic bacteria associated with BV are known to upregulate pro-inflammatory cytokines, which leads to an increase in prostaglandins(PG). Probiotic lactobacilli have been shown to reverse BV and the GG and GR-1 strains of lactobacilli *rhamnosus* are known to downregulate pro-inflammatory cytokines in mouse macrophages *in vitro*. We hypothesized that *Lactobacillus rhamnosus* GR-1 will interfere with the cascade leading to PG synthesis by downregulating pro-inflammatory cytokine production and COX_2 protein expression in human placental trophoblast cells.

Methods: Term placentae were collected from women undergoing elective Caesarean section. Placental trophoblasts were isolated and incubated for 72h. Cells were serum starved for 12h and divided to four groups: 1)No treatment, 2)Treatment with LPS (200 ng/ml for protein measurements or 100 ng/ml for cytokine measurements) after a further 12h, 3)Treatment with the supernatant from lactobacilli cultures(1:20 dilution) for 12h, 4)Pretreatment with lactobacilli supernatant for 12h and subsequent treatment with LPS. Protein was extracted and media collected after 8h. COX_2 expression levels were measured by Western Blot analysis and TNF-α and IL-1β concentrations measured by ELISA.

Results: LPS stimulation caused a marked increase in TNF-α production by placental trophoblasts(57.5±6.1 to 1609.3±612.6 pg/ml, $p<0.05$). Pretreatment with lactobacilli supernatant completely abolished this increase(148.4±43.5 pg/ml, $n=7, p<0.05$). LPS also caused a significant increase in COX_2 expression. Pretreatment with lactobacilli supernatant downregulated this expression by 21% ($p<0.05, n=8$). Treatment with lactobacilli supernatant alone had no effect on cytokine production or COX_2 expression. There were no changes in IL-1β concentrations with any treatment.

Conclusion: Probiotic lactobacilli inhibit both TNF-α production and COX_2 expression in placental trophoblast cells *in vitro*. This study provides evidence for a potential mechanism by which probiotic lactobacilli may reduce the risk of PTB in women with BV.

302

Amniotic Fluid Interleukin-6 Increase Is an Indicator of Preterm Birth in Caucasians but Not in African-Americans. Ramkumar Menon,[1] M Constanza Camargo,[2] Stephen J Fortunato.[1] (SPON: Kelle H Moley). *[1]The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN, USA; [2]Department of Medicine, Vanderbilt Uinversity, Nashville, TN, USA.*

OBJECTIVE: The disparity in the preterm birth (PTB) rate between African-Americans (AA) (17.6%) compared to Caucasians (C) (10.7%) is high.The cause of this ethnic disparity is unclear and can not be explained by known biologic/etiologic factors. Higher concentrations of TNF-α and interleukin (IL)1β in AA and higher IL8 in C PTB derived amniotic fluid (AF) providing evidence for disparity in the inflammatory response associated with PTB have been reported. Herein we examine IL6, which is reported as a "marker" of PTB in the AF of AA and C women.

METHODS: In this case (PTB ≤ 36 weeks gestation) control (normal term delivery > 37 weeks) study 321 AF samples were collected (147 cases [49 AA and 98 C] and 174 controls [85 AA and 89 C]) at the time of active labor. AF IL6 concentration was measured by ELISA. Median differences (pg/ml) between cases and controls were examined by Wilcoxon Ranked test. Using unconditional logistic regression analysis the odds ratios (OR) for PTB were calculated based on the distribution of IL6 among controls.

RESULTS: In the pooled data (AA+C), the median IL6 concentration was significantly higher in cases (3068) than in controls (1863 pg/ml; p=0.007). When data were stratified by race, a significant difference was observed in C cases compared to controls (3773 vs. 1682, respectively; p=0.0003), but not in AA cases compared to their respective controls (2042 vs. 2366, respectively; p=0.59). In combined (AA+C) multivariate analysis, when the highest (>5310) and the lowest (<844) quartiles of IL6 were compared, the adjusted OR for PTB risk was 2.7 (95% CI: 1.3 – 5.4; p trend = 0.003). In C, ORs (95% CI) for PTB across quartiles were 1.74 (0.62 - 4.88), 1.09 (0.39 - 3.02), and 5.68 (2.15 - 15.0; p trend = 0.003). No such association was found between IL6 concentrations and PTB risk in AA.

CONCLUSIONS: Contrary to previous studies reporting a significant association between increased IL6 concentration and PTB risk, this study suggests that there are ethnic-specific associations. Elevated IL6 concentrations significantly increase the PTB risk in C, but not in AA suggesting that proinflammatory cytokine response in PTB is not generalizable. The pathophysiologic pathways of PTB may differ in different ethnic groups and these differences may contribute to rate disparity.

303

The Modulation of MCP-1 Expression during LPS-Induced Preterm Delivery in the Pregnant Mouse. Mark Phillippe,[*] Allaire Diamond, Karren Oppenheimer, Leigh Sweet. *Department of Obstetrics & Gynecology, University of Vermont College of Medicine, Burlington, VT, USA.*

Objective: Infection/inflammation-induced preterm delivery is associated with a robust increase in proinflammatory cytokines and chemokines, including monocyte chemoattractant factor-1 (MCP-1). In human pregnancies, elevated MCP-1 in serum and uterine tissue has been reported during pregnancies complicated by preterm labor. The origin of the elevated MCP-1 levels, however, is unclear. The current studies sought to characterize the expression of MCP-1 in the pregnant uterus and other organs during intrauterine LPS-induced preterm delivery (PTD) in the mouse.

Methods: Using sterile surgical technique, day-15 pregnant CD-1 mice underwent intrauterine injection of 250 µg LPS. Subsequently, 10-14 mice for each group were euthanized at 0, 2, 6, 12, 18 and 24 hours after LPS injection. Uterus and other mouse organs were harvested, placed in RNAlater or frozen in Tissue-Tek OCT compound. Total RNA was isolated using the Trizol reagent; genomic DNA was removed using TURBO DNA-free. cDNA was made using iScript cDNA Synthesis Kit. Qualitative MCP-1 expression was determined using the iTaq DNA polymerase kit and mouse specific sense and antisense primers. Real-time quantitative RT-PCR was performed in an ABI Prism 7000 multicycler using the Power SYBR Green PCR Master Mix. RT-PCR data were normalized using 3 constitutively expressed genes. Immunohistochemical studies were performed on 25 µm sections of pregnant uteri using anti-MCP-1 polyclonal antibodies and the Vectastain Elite ABC kit.

Results: With this model, PTD is rare before 6 hours (only 7.7%); whereas, by 12 hours 71.4% and by 18 hours 91.7% had delivered. Qual-RT-PCR confirmed MCP-1 mRNA expression in uterus, lung, kidney and liver. Real-time quant-RT-PCR demonstrated a 75 fold increase in MCP-1 mRNA in uterine tissue at 2 hours, 46 fold at 6 hours, and 8 fold at 12 and 18 hours. MCP-1 mRNA also increased markedly in kidney (260 fold) and liver (185 fold) at 2 hours; and less in lung (12 fold). The immunohistochemistry studies confirmed MCP-1 protein expression within the pregnant mouse uterus.

Conclusions: These studies have confirmed robust expression of MCP-1 mRNA within uterus and other mouse tissues during intrauterine LPS-induced PTD. The temporal relationship of these events and the magnitude of the MCP-1 response suggest that these events could be mechanistically related. (Funded by NIH HD044747)

304

Inflammation-Induced Preterm Delivery and Plasminogen Activator Inhibitor-1 (PAI-1) Expression in the Pregnant Mouse. Mark Phillippe,* Allaire Diamond, Karen Oppenheimer, Leigh Sweet. *Department of Obstetrics & Gynecology, University of Vermont College of Medicine, Burlington, VT, USA.*

Objective: The systemic inflammatory response syndrome (SIRS) is associated with the activation of coagulation and fibrinolysis, due in part to increased plasminogen activator inhibitor-1 (PAI-1). In addition to its role as an inhibitor of plasminogen activators, PAI-1 appears to have cellular effects including modulation of cell motility, proliferation, metabolism, and stimulation of fibrosis. The current studies sought to characterize the expression of PAI-1 (in comparison to tissue factor (TF)) in the pregnant uterus and other organs during intrauterine LPS-induced preterm delivery (PTD) in the mouse.

Methods: For these studies, day-15 pregnant CD-1 mice underwent intrauterine injection of 250 µg LPS. Subsequently, 10-14 mice were euthanized at 0, 2, 6, 12, 18 and 24 hours after LPS injection. Uterus and other mouse organs were harvested, placed in RNAlater or frozen in Tissue-Tek OCT compound. Total RNA was isolated using the Trizol reagent; genomic DNA was removed using TURBO DNA-free. cDNA was made using iScript cDNA Synthesis Kit. Qualitative PAI-1 and TF expression was determined using the iTaq DNA Polymerase kit and sense and antisense primers for these two mouse genes. Real-time quantitative RT-PCR was performed in an ABI Prism 7000 multicycler using the Power SYBR Green PCR Master Mix. RT-PCR data were normalizing to 3 constitutively expressed genes. Immunohistochemical studies were performed on 25 µm sections of pregnant uteri using the Vectastain Elite ABC kit with anti-PAI-1 and anti-TF polyclonal antibodies.

Results: Qual-RT-PCR confirmed PAI-1 and TF mRNA expression. Quant-RT-PCR demonstrated a 25 fold increase in PAI-1 mRNA in uterus at 2 and 6 hrs; in contrast, TF mRNA decreased markedly (22.3% at 6 hours, 13.6% at 12 hours and 2-3% at 18-24 hours) after LPS injection. PAI-1 mRNA also increased in lung (88 fold), kidney (100 fold) and liver (380 fold) at 2 hrs. TF mRNA expression decreased in liver; and increased <2 fold in lung and kidney. The immunohistochemistry studies confirmed PAI-1 and TF protein expression the pregnant mouse uterus.

Conclusions: These studies have confirmed robust PAI-1 mRNA expression within uterus and other organs during inflammation-induced PTD; events similar to SIRS. These studies provide support for the potential relationship between inflammation and coagulation during PTD. (Funded by NIH HD044747)

305

Upregulation of IL-8 Synthesis by IL-1β and IL-6 (NF-IL6) in Combination. Shirin Khanjani, Yun S Lee, Vasso Terzidou, Mark R Johnson,* Suren R Sooranna, Phillip R Bennett.* *Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, London, United Kingdom.*

IL-8 is a chemokine whose expression within the uterus increases dramatically with the onset of labour and plays an important role in cervical remodeling and probably in contractions. We have previously shown that overexpression of either NFκB p65 or C/EBP LAP alone leads to a 2 fold induction in IL-8 promoter activity in human myocytes. NFκB p65 or C/EBP LAP in combination are synergistc causing a 6 fold induction. IL-1b acts principally to increase NFκB activity whilst IL-6 acts through C/EBP. We have extended these experiments to examine the effect of overexpression of NFκB p65 and C/EBP and of IL1-b and IL-6 upon IL-8 protein synthesis.

Human myocytes in culture were transfected with expression vectors for NFκB p65 or C/EBP LAP alone, or the two in combination. Initial western analysis studied were performed to determine the time of peak expression of proteins expressed via transfected vectors. Myocytes were found to express both NFkB and C/EBP without overexpression. Overexpression was seen to peak at 24 to 36 hours. Cultures were stopped at between 8 and 72 hours post transfection and culture medium assayed for IL-8 by ELISA. In separate experiments myocytes were incubated with IL-1b (1ng/ml) and IL-6 (NF-IL6, 10ng/ml) alone or in combination and IL-8 concentrations in the medium were measured at 6 and 12 hours.

Overexpression of NFκB p65 increased IL-8 synthesis by 2, 18, 28, and 50 fold at 24, 36, 48 and 72 hours respectively. Overexpression of C/EBP LAP caused a much smaller increases of 3 and 5 fold at 48 and 72 hours. The combination of NFkB p65 and C/EBP LAP caused a synergistic increase of 60 fold only at 72 hours. Incubation of myocytes with IL-6 alone had no significant effect upon IL-8 synthesis whilst IL1b caused a 15 fold increase at 6 hours and 12 hours. Incubuation with IL1b and IL-6 in combination led to 26 and 75 fold increases in IL-8 synthesis at 6 an 12 hours respectively.

These data suggest that the large increases in IL-8 synthesis within the uterus seen in association with labour are likely to be due to the combined effects of IL1b and IL-6. The substantially larger increase in IL-8 synthesis seen at the later time point may reflect the later endogenous activation of C/EBP caused by IL-6. The interaction between NFκkB and C/EBP appears to be important in the regulation of the onset of labour.

306

Causal Pathway Modelling of mRNA Data Supports Inflammatory Drive towards Labor Onset. Andrew Bisits,[1] Roger Smith,*[1] David A MacIntyre,[1] Kenneth Kwek,[2] George Yeo,[2] Kellie Ann Taylor,[1] Eng-Cheng Chan.[1] *[1]Obstetrics and Gynecology, John Hunter Hospital, NSW, Australia; [2]KK Women's and Children's Hospital, Singapore.*

Background: Evidence from many sources suggests that inflammation plays a key role in the progress towards the onset of normal human labor. However, the traditional experimental approach to hypothesis testing is difficult in the pregnant woman.

Hypothesis and Aims: To test the hypothesis that inflammation drives labor using formal causal modeling to infer plausible causal networks amongst mRNA data for key genes obtained from term myometria.

Methods: Term myometrial samples (n=69) were obtained at elective Caesarean section. Real time PCR was used to quantify the expression of genes that we found by Suppression Subtractive Hybridisation to be altered by labor (matrix metalloproteinases 2, 3 and 9; interferon-induced transmembrane protein 2, oxytocin receptor, interleukin 8, Manganese superoxide dismutase, triosephosphate isomerase 1, nucleophosmin, testin, fibronectin 1, SerpinF1, prostaglandin E receptors 1 and 2, prostaglandin F receptor) and other genes associated with parturition (progesterone receptors total and B, estrogen receptor alpha, COX2, connexin43, NFkB p50 and p65 subunits). Principal Components Analysis was performed on the entire data set and a clear distinction between NL and L samples was evident. Subsequent analysis focused on NL samples only. Correlations amongst the data were checked using STATA (v9) and the data grouped into 5 latent variables based on the correlation matrix and *a priori* biological knowledge. The latent variables consisted of a steroid-related group (Steroids; estrogen receptor and a measure of progesterone receptors), two separate inflammatory factors, (a) NFkB-related and (b) IL8- and COX2-related factors, CX43-related and OTR-related factors. To maximize statistical power, latent variables were converted to composite scores which were used for the formal process of inferring biologically plausible inter-relationships using Tetrad and D-Graph.

Results. The biologically plausible structure that statistically was most consistent with our data (Tetrad P=0.7, D-Graph P=0.73) was a model where inflammatory factors drive changes in steroid receptors, and steroid receptors and oxytocin-related factors drive changes in connexin 43-related genes.

Conclusion. These results support the hypothesis that inflammatory factors drive myometrial connectivity near the time of human labor onset.

307

MPA Blocks Effects of LPS-Induced Preterm Birth on Ripening, Immigration of Immune Cells, and Density of Nerve Fibers in the Cervix. Steven M Yellon,[1] Charlotte A Ebner,[1] Michal A Elovitz.*[2] *[1]Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA; [2]Dept Ob/Gyn; Center for Research in Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: Inflammatory processes are implicated in cervical ripening both in human term and preterm parturition (*JSGI 10:323, 2003*). In mice, inflammatory pathways are activated in the cervix prior to preterm delivery (*JSGI 12:137A,2005*). Whether progestational agents can modify these pathways as a mechanism for prevention of preterm birth needs to be elucidated. Therefore, studies were performed to determine 1) whether inflammation-induced preterm cervical ripening involves trafficking of immune cells or hypertrophy of innervation, and 2) whether a progestational agent alters these pathways.

Methods. On day 15 of gestation CD-1 mice (n=3/group) received an intrauterine injection of saline (Sal), lipopolysaccharide (LPS), medroxyprogesterone (MPA) alone, or MPA prior to LPS. Cervices were obtained 6 h later; tissues were fixed, sectioned, and processed to stain collagen structure or to identify macrophages and neutrophils and nerve fibers (*Biol Reprod 61: 879,1999; JSGI 12: 578, 2005*).

FRIDAY

Results. In comparison to Sal controls, LPS induced cervical ripening as measured by optical density of polarized light (P<0.001). MPA pretreatment blocked this reduction in collagen structure. Although the number of resident macrophages declined after LPS relative to that in Sal group, a significant increase in resident neutrophil was evident (P=0.01). MPA forestalled the emigration of macrophages and immigration of neutrophils. LPS did not alter the density of peripherin-stained nerve fibers, however, MPA reduced innervation in the cervix.

Conclusions: Intrauterine inflammation promotes cervical ripening as assessed by changes in collagen structure, immigration of neutrophils, and emigration of macrophages. The role of neural activity in this inflammatory process, rather than density of innervation, remains a consideration. Progestogen treatment forestalled inflammatory-associated changes. Thus, inflammation-induced preterm birth may differ from recruitment of immune cells and hypertrophy of innervation associated with parturition at term. These data raise the possibility that progesterone may regulate traffic of immune cells and proinflammatory mediators that are proposed to ripen the cervix early in the process leading to preterm birth.

308

LPS-Induced Preterm Delivery Is Not Critically Dependent on T or B Cells. Peyman Bizargity, Leigh M Sweet, Mark Phillippe,* Elizabeth A Bonney.*
OB/GYN, University of Vermont College of Medicine, Burlington, VT, USA.

Introduction: Preterm delivery persists as a major clinical problem. Current thinking is that infection and inflammation contribute greatly to the disease. However, the role of T and B cells as positive or negative regulators of this process is unclear.

Objective: To determine if absence of T and B cells changes susceptibility to LPS-induced preterm labor in a mouse model.

Method: C57BL/6 *rag*-1$^{-/-}$ (no T or B cells, RAG-KO) and wild type C57BL/6 (WT) females were mated with same-strain males. On day 15 post coitus they were injected intraperitoneally with 0.6 to 30 µg of LPS. Twenty-four hours later the mice were euthanized and examined for delivery of pups. LPS-induced delivery rates were analyzed by Chi-Square. In a separate experiment, day 15 pregnant RAG-KO and WT mice were injected with 3µg LPS or PBS and euthanized either 6 or 24 hours later (n=2 per strain per time point). Peripheral blood was analyzed for cytokine release by Bio-plex cytokine immunoassay. Assay values from LPS-injected mice minus the average value obtained from PBS injected mice were compared.

Results: LPS injection led to dose-dependent preterm delivery in RAG-KO mice, with 8/8, 3/3, 3/3, 8/9, and 1/3 mothers delivering 24 hours after injection of 30, 10, 5, 3 and 0.6µg LPS/mouse, respectively. While WT mice given 30µg of LPS delivered prematurely (7/7), those given 3µg of LPS did not (0/8, p<0.0002), suggesting an increased susceptibility in RAG-KO mice.

At 6 hours after injection with 3µg LPS, peripheral blood from day 15 RAG-KO mice had increased IL-1 alpha (12,307; 7041 pg/ml), IL-6 (13,000;12,995 pg/ml), IL-12 p40 (17,608; 8,596 pg/ml), IL-13 (2224;1846g/ml), EOTAXIN (17073; 15383 pg/ml), G-CSF(84865; 87709 pg/ml), KC (53179; 40840 pg/ml), MCP-1(32336; 28286 pg/ml), MIP-1b (8683; 4544 pg/ml) and RANTES (19746; 19165 pg/ml) similar to previous reports in normal mice. At 24 hours, these cytokines remained elevated, particularly G-CSF (81636; 23628 pg/ml) and MCP-1(1633; 2581 pg/ml). In contrast WT mice expressed lower IL-6 at 6 hours (5974; 314 pg/ml) and at 24 hours expressed lower IL-6 (121; 169pg/ml) and MCP-1(425; 1105 pg/ml).

Conclusion: T and B cells are not critical for LPS-induced preterm delivery and mice deficient in T and B cells are more susceptible. This is correlated with increased cytokine release, and may be secondary to altered down regulation of the LPS response or altered clearance of LPS. *Supported by NIH-HD044747.*

309

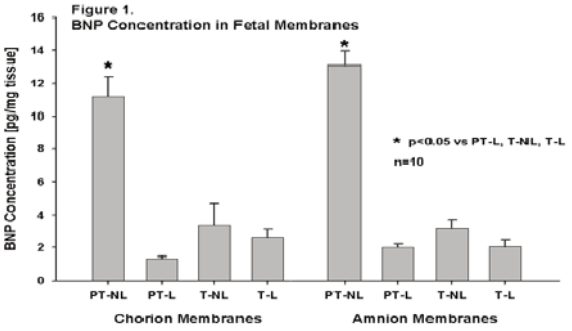
Premature Decrease of Brain Natriuretic Peptide (BNP) Production by Fetal Membranes May Cause Preterm Labor. Jorge A Carvajal,*[1] Ana M Delpiano,[1] Mauricio A Cuello,[1] Jose A Poblete,[1] Carl P Weiner.*[2] [1]*Obstetricia y Ginecología, Pontificia Universidad Católica de Chile, Santiago, RM, Chile;* [2]*Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*

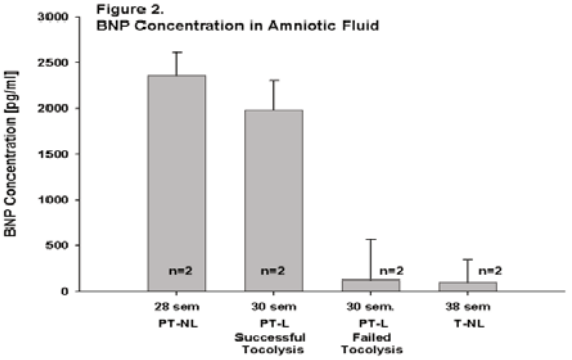
Objective: Paracrine regulation of myometrial quiescence has been shown in the human. We found that BNP is synthesized by human fetal membranes (hFM) and inhibits oxytocin-induced contractions of preterm human myometrium. Presently, we sought to test aspects of the hypothesis that a premature decrease in fetal membrane released BNP was associated with idiopathic preterm labor (PT-L).

Methods: hFM were obtained from term and preterm cesarean deliveries, either in labor or not labor (n=10 each group), and BNP synthesis and release measured by RIA and RT-PCR. BNP release was based on the levels after a 30min incubation of FM in media (CM). We also quantitated BNP in human amniotic fluid (AF) samples (n=2 each group).

Results: BNP and BNP mRNA were of similar concentrations in amnion and chorion. There was more BNP in the membranes compared to CM. BNP and BNP mRNA were significantly higher in FM and CM from preterm not in labor compared to term not in labor samples. There was a significant reduction of BNP and BNP mRNA in FM obtained from preterm labor patients (Figure1). Further, BNP was increased in preterm compared to term AF (both not in labor). BNP concentration was lower in AF from PT-L patients that failed tocolysis compared to those with successful tocolysis (Figure2).

Conclusions: The larger concentration of BNP in preterm samples absent labor and its decrease in samples from women in labor suggests a role for BNP in myometrial quiescence. These results tantalizingly suggest that if the AF BNP is low, current tocolytic agents will not successfully delay delivery. This study was supported by a grant from Chilean Government Fondecyt 1020675.





310

Changes in the Cervical Epithelium during Cervical Ripening and Parturition. Brenda C Timmons, Mala S Mahendroo.* *OBGYN, University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Objective- Cervical epithelia have numerous functions that include proliferation, differentiation, maintenance of fluid balance, protection from environmental hazards, and paracellular transport of solutes via tight junctions. Epithelial functions must be tightly regulated during pregnancy and parturition as the cervix undergoes extensive growth and remodeling. The objective of this study was to evaluate tight junction proteins as well as markers of epithelial cell differentiation in wildtype (WT) and cervical ripening defective steroid 5a-reductase type 1 deficient mice (*Srd5a1*$^{-/-}$) to better understand how the permeability barrier is regulated during pregnancy and parturition.

Methods- Quantitative real time PCR and Western blot analysis were performed to identify changes in mRNA and protein expression, respectively, of genes involved in apical junction complexes (APC) and epithelial cell differentiation during pregnancy and parturition in the cervix. Immunofluorescence was carried out on cervical sections to visualize changes in the localization of the tight junction proteins claudin 1 and claudin 2. Gene and protein expression were also measured in the *Srd5a1*$^{-/-}$ mouse which fails to undergo cervical ripening due to inadequate local progesterone metabolism.

Results- While numerous tight junction proteins are expressed in the nonpregnant cervix, claudin 1 and 2 are temporally regulated in pregnancy. Claudin 1 expression is increased at term while claudin 2 expression declines at term. The cellular localization of claudin 1 shifts at the end of pregnancy (gestation d18.75) to the plasma membrane in a lattice pattern consistent with tight junctions in the apical cells. The timing of claudin 1 enriched tight

junctions coincides with initiation of terminal differentiation of cervical squamous epithelia as evidenced by the increased mRNA expression of genes expressed by differentiated epithelia late on gestation d18. The cervical ripening defective mouse *Srd5a1*$^{-/-}$ which has an elevated local progesterone concentration also has aberrant claudin 1 and 2 expressions, fails to form claudin 1 enriched tight junctions, and lacks normal expression of genes involved in epithelial terminal differentiation.

Conclusion- These data suggest that changes in cervical permeability barrier properties during pregnancy are in part negatively regulated by progesterone and that dynamic changes in barrier properties of the cervix occur during pregnancy and parturition.

311

Thrombin and Vascular Endothelial Factor Activation of Endometrial Endothelial Cells. A Potential Pathway towards IUGR. Graciela Krikun,[*] ST Joseph Huang, Frederick Schatz,[*] Carolyn Salafia,[*] Charles J Lockwood.[*] *Ob/Gyn and Rep. Sciences, Yale University, School of Medicine, New Haven, CT, USA.*

Introduction: IUGR and abruption +/- fetal loss are associated with reduced uteroplacental blood flow, decidual vasculopathy, endothelial cell dysfunction, thrombosis, inflammation and hemorrhage. We posit that reduced uteroplacental blood flow causes focal decidual hypoxia that generates VEGF. The latter acts directly on decidual endothelial cells to induce aberrant expression of tissue factor **(TF)** which in turn generates thrombin to further TF expression and inflammatory cytokines. Both VEGF and TF induce aberrant angiogenesis/ vessel maintenance and induction of a prothrombotic surface causing both the decidual hemorrhage and thrombosis observed in these adverse pregnancy outcomes. This hypothesis is supported by our findings below.

Methods: Twenty five cases and controls (gest. age 37-40 weeks), were collected after receiving written informed consent. Quantification of IHC for TF was conducted following image capture with the Aperio T3 instrument. All cell culture was as previously described in our laboratory.

Results: Our findings demonstrate aberrant TF expression in decidual vessels from pregnancies complicated by IUGR and stillbirth. Specifically, basal plate uteroplacental vessel segments from cases of low birth weight with villous evidence of maternal uteroplacental malperfusion had increased percent of strong positive endothelial immunostaining for TF (15±3%) *vs* cases of low birth weight associated with chronic villitis (5±4%) or controls (2±2%). *In vitro,* treatment of endometrial endothelial cells with VEGF or thrombin induced TF protein and mRNA expression. Quantitative RT-PCR indicates that thrombin enhances (> 10-fold) the output of several inflammatory cytokines in these cultures. The greatest effect (> 2-log) was seen on macrophage inducing protein 3α. In addition, thrombin results in aberrant tubulogenesis reflected by aggregate endothelial cell formation on a 3D cell culture system. Most importantly, while co-culture of trophoblastic cells with endothelial cells demonstrated the invasion of and maintenance of tubular structures, addition of thrombin had adverse effects.

Conclusion: We posit that reduction in uteroplacental flow can initiate a cascade of molecular effects leading to hypoxia, abnormal vascular TF expression, thrombosis, inflammation, and aberrant vessel maintenance resulting in untoward pregnancy outcomes.

312

Effect of Vitamin C and TNF$_\alpha$ on Fetal Membrane Strength. MM Perez-Fournier,[1] RM Moore,[1] D Kumar,[1] S Yousfi,[1] B Stetzer,[2] Brian Mercer,[*2] JM Mansour,[3] John Moore.[*1,2] *[1]Pediatrics; [2]Reproductive Biology; [3]Aerospace and Mechanical Engineering, Case Western Reserve University, Cleveland, OH.*

BACKGROUND: Preterm premature rupture of membranes (PPROM) is associated with 30% to 40% of premature deliveries and subsequent significant neonatal morbidity and mortality. Term fetal membranes (FM) have been recently demonstrated to undergo a weakening process in the regional zone overlying the cervix characterized by collagen remodeling and apoptosis. Although it is not known, it is suspected that PPROM undergo a similar process. We have recently demonstrated that TNF$_\alpha$ incubated with FM, *in vitro*, causes biomechanical weakening and the same biochemical changes seen in the weak zone of term FM. In PPROM, there is evidence of increased collagenolytic activity with parallel increases in TNF$_\alpha$ concentrations. As vitamin C (VC) is critical for several of the biochemical steps in the synthesis and maintenance of collagen, it has been suggested as a preventive therapy for PPROM. Although numerous basic and clinical studies support this idea, there are also a number of reports of increased cellular apoptosis with VC. There have been no studies directly examining the effect of VC on FM strength.

HYPOTHESIS: We hypothesize that vitamin C will prevent TNF$_\alpha$ induced FM weakening.

METHODS: Fetal membranes obtained after term, uncomplicated, repeat-Caesarean deliveries were selected for study. Full-thickness FM fragments were cultured with or without VC [1 mM], and with or without TNF$_\alpha$ [50 ng/ml]. Physical properties were then examined with specially adapted industrial rupture strength testing equipment after 48 hours of incubation.

RESULTS: FM incubated with VC or TNF$_\alpha$ alone each exhibited statistically significant decreases in strength compared with controls. ($p = 0.035$ and $p= 0.039$ respectively). The combination of VC and TNF$_\alpha$ cultured FM exhibited a further decrease in strength compared with controls ($p = 0.004$).

CONCLUSION: Contrary to our hypothesis, VC and TNF$_\alpha$ administered *in vitro*, at term, both cause significant weakening of cultured FM. The combination was additive in its weakening effect. Caution should be exercised in utilizing high dose VC to prevent PPROM. Support NIH HD048476 to JJM.

313

Proteomic Assessment of Mechanically Strong vs. Weak Amnion Fragments from Vaginally Delivered Fetal Membranes. R Moore,[1] E Yohannes,[2] M Chance,[2] D Kumar,[1] Brian Mercer,[*3] John Moore.[*1,3] *[1]Pediatrics; [2]Proteomics Center; [3]Reproductive Biology, CWRU, Cleveland, OH.*

Rupture of the fetal membranes (FM) involves programmed processes of biochemical and physical weakening prior to, and during, labor. In an effort to screen for proteomic differences between weak and strong regions of the FM following labored vaginal delivery, 2-dimensional gel electrophoresis (2-DIGE) was used to separate and compare protein fingerprints of strong and weak amnion fragments after topographical mapping and rupture testing.

Full-thickness FM fragments from vaginally delivered fetal membranes were strength tested. Fragments were separated into groups with rupture strength: (A)<4.4, (B)≥ 4.4 and <8.8 and (C)≥8.8 newtons. Amnion was stripped from the chorion of each fragment and pooled amnion fragments from the weakest (A) and strongest (C) groups were processed for 2-DIGE, and differential, in-gel analysis. MW and PI data for significantly up/down-regulated proteins were researched using ExPASy Proteomics Server Tools. Candidate proteins were screened by western blot.

2671 resolved protein spots were detected when comparing the strongest and weakest amnion fragments. Of these, 29 proteins showed -1.5, and lower, fold differences while 35 proteins showed 1.5, and higher, fold changes. 5 proteins showed more than a two fold decrease while 9 proteins showed greater than two fold increase. A cluster of 4 proteins with greater than 2 fold change in the 30-50kD MW/2.5-4.5 PI range was selected for initial study. TagIdent analysis identified 41 suspect proteins in this range. Western blot analysis confirmed Fibulin-5 expression was decreased by 45% (2.16 ± 0.5 vs 1.28 ± 0.3 OD, $p<0.0001$) in weak FM fragments.

We have previously shown that a weak zone exists in topographically mapped fetal membranes from both vaginally delivered and C-sectioned patients and that these weak zones exhibit biochemical changes including increases in MMP9 and apoptosis, and decreased TIMP-3 protein.

Here, we have differentially screened amnion fragments from the strong and weak zones of vaginally delivered, term, FM and identified differentially expressed proteins. Fibulin-5, a regulator of elastic fiber formation, was found to be depressed in weak zones of the FM. Further characterization and identification of this, and additional proteins by MALDI Mass Spec analysis and western blotting may provide additional insight into the mechanisms of FM weakening and rupture. Support NIH HD048476.

314

17-α-Hydroxyprogesterone Caproate and Thrombin-Induced Expression of Contraction Associated Proteins in Term Gestational Myometrium. David N Hackney,[1,2] Jye-Ping Chiao,[1,2] Suzanne E Peterson,[1,2] Hyagriv N Simhan.[*1,2] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetric-Fetal Pharmacology Research Units Network, Bethesda, MD, USA.*

Objective: 17-α-hydroxyprogesterone caproate (17-OHPC) has clinically been demonstrated to reduce the prevalence of preterm birth (PTB) through a mechanism which is not clear. Many cases of PTB are related to clinical bleeding events, presumably through the activation of thrombin. Thrombin has been demonstrated to stimulate the expression of contraction associated proteins (CAPs) in term gestational myometrium. The hypothesis of this study is that 17-OHPC alters the increased expression of oxytocin receptor (OTR), COX-II and Connexin-43 in myometrial cells exposed to thrombin.

FRIDAY

Methods: Samples of myometrial tissue were collected from pregnant patients at term undergoing cesarean sections. After tissue cultures were established, the cells were incubated with either varying concentrations (1-100ng/mL) of 17-OHPC alone, 1 U/mL of thrombin or thrombin and 17-OHPC. The relative protein concentrations of OTR, Cox-II and Connexin-43 were then determined through Western blot analysis. The results were normalized to control.
Results: Table 1 contains the relative concentrations of the CAPs in the different sample groups, normalized to control. No statistically significant differences were noted between the groups, with the exception of an increased expression of COX-II among samples exposed to 17-OHPC alone (p=0.03).
Conclusion: 17-OHPC does not effectively reduce the thrombin stimulated expression of CAPs in term gestational myometrial cells in tissue culture. Decreasing the expression of CAPs in patients with pathological thrombin activation is thus unlikely to explain the clinical effects of 17-OHPC in the prevention of PTB.

CAP expression normalized to control

Exposure:	Thrombin (1 U/mL)	17-OHPC	Thrombin+17-OHPC
OR	1.58	1.92	1.46
COX-II	1.91	4.23	3.0
Connexin-43	1.21	1.21	2.06

315

Single Nucleotide Polymorphisms in the Macrophage Migration Inhibitory Factor (MIF) Gene and Spontaneous Preterm Birth. Felice Arcuri,[1] Paulo Toti,[1] Thomas Morgan,[2] Victoria Snegovskikh,[3] Edward Kuczynski,[3] Hee Joong Lee,[3] Frederick Schatz,[*3] Se-Te Joseph Huang,[3] Catalin Buhimschi,[3] Edmund Funai,[*3] Irina Buhimschi,[3] Charles Lockwood,[*3] Christian Pettker,[3] Errol Norwitz.[*3] *[1]Human Pathology and Oncology, University of Siena, Siena, Italy; [2]Pediatrics, Washington University, St. Louis, MO; [3]Ob/Gyn, Yale University, New Haven, CT.*
OBJECTIVE: MIF is highly expressed at the maternal-fetal interface and inhibits natural killer cell activity in the uterus. Aberrant levels of MIF are associated with miscarriage and preeclampsia. Single nucleotide polymorphisms (SNPs) in the human MIF gene have been associated with rheumatologic diseases, but their association with preterm birth has not been previously examined. This study investigates the association between -173(C>G) and +656(C>G) SNPs in maternal MIF gene and preterm birth.
METHODS: Consecutive patients with spontaneous (non-iatrogenic) preterm birth were identified from the March of Dimes PERI project (MOD #20-FY03-30) at New York University, NY and Yale University, CT from Jan 1989-June 2005. Cases were matched (4:1) with uncomplicated term deliveries (controls). DNA was extracted from stored buffy coats and genotyping performed using established primers. All specimens were linked with demographic and medical data abstrated from maternal/neonatal records. Genotyping was performed using the Sequenom MALDI-TOF platform with individualized assay designs created by automated Spectrodesign software (Sequenom, San Diego, CA). Data were analyzed by permutation-based exact chi-square test for 2x3 genotype tables and by logistic regression analysis with adjustment for race (SPSS, Chicago, IL).
RESULTS: Samples were successfully genotyped for -173(C>G) and +656(C>G) in 98.5% and 99.5% of cases, respectively. Hardy-Weinberg chi-square genotype distribution expectations were met for -173 (C>G) cases (p=0.63) and controls (p=0.06), and +656(C>G) cases (p=0.74) and controls (p=0.17). In logistic regression analysis with adjustment for race, there was no significant association between preterm birth and either -173(C>G) [cases CC=8, CG=43, GG=36; controls CC=36, CG=135, GG=228 (p=0.203)] or +656(C>G) [cases CC=52, CG=31, GG=7; controls CC=247, CG=127, GG=27 (p=0.794)].
CONCLUSION: The -173(C>G) and +656(C>G) SNPs in the human MIF gene are not associated with spontaneous preterm birth. Further studies are required to better define the role of MIF in human pregancy.
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316

Single Nucleotide Polymorphisms in the Human Progesterone Receptor (PR) Gene and Spontaneous Preterm Birth. Errol Norwitz,[*1] Thomas Morgan,[2] Victoria Snegovskikh,[1] Edward Kuczynski,[1] Hee Joong Lee,[1] Frederick Schatz,[*1] Se-Te Joseph Huang,[1] Catalin Buhimschi,[1] Edmund Funai,[*1] Irina Buhimschi,[1] Antonette Dulay,[1] Charles Lockwood.[*1] *[1]Ob/Gyn, Yale University, New Haven, CT; [2]Pediatrics, Washington University, St. Louis, MO.*
OBJECTIVE: Progesterone supplementation can prevent preterm birth in some high-risk women. Progesterone acts by binding to PR and modulating expression of target genes. The +770(C>T) and +660(G>T) single nucleotide polymorphisms (SNPs) in the single-copy PR gene are associated with recurrent miscarriage and reproductive tract malignancies. This study investigates the association between these two SNPs in the maternal PR gene and preterm birth.
METHODS: Consecutive patients with spontaneous preterm birth were identified from the March of Dimes PERI project (#20-FY03-30) at NYU, NY and Yale, CT from Jan 1989-June 2005. Cases were matched (4:1) with uncomplicated term deliveries (controls). DNA was extracted from stored buffy coats and genotyping performed using established primers. All specimens were linked with demographic and medical data abstrated from maternal/neonatal records. Genotyping was performed using Sequenom MALDI-TOF platform with individualized assay designs created by automated Spectrodesign software (Sequenom, San Diego, CA). Data were analyzed by permutation-based exact chi-square test for 2x3 genotype tables and by logistic regression analysis with adjustment for race (SPSS, Chicago, IL).
RESULTS: Genotyping was successful in 99.4% of samples. Hardy-Weinberg chi-square expectations were met for +770 cases (p=0.23)/controls (p=0.21) and +660 cases (p=0.3)/controls (p=0.78). Genotype distribution for +770 cases (n=94) were: CC=75 (79.8%), CT=16 (17.0%), TT=3 (3.2%); controls (n=401): CC=317 (79.1%), CT=75 (18.7%), TT=9 (2.2%). Consistent with strong linkage disequilibrium between these SNPs, the genotype distributions of +770 resembled that of +660 cases (n=95): GG=75 (78.9%), GT=17 (17.9%), TT=3 (3.2%); and controls (n=401): GG=314 (78.7%), GT=76 (19.0%), TT=9 (2.3%). These data are consistent with prior reports. In logistic regression analysis with adjustment for race, there was no difference in +660 (p=0.99) or +770 (p=0.80) genotypes in cases vs controls.
CONCLUSION: The +770(C>T) and +660(G>T) SNPs in the human PR gene are not associated with spontaneous preterm birth. Further studies are required to identify women who may benefit from progesterone supplementation.
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317

Binding Characteristics of Barusiban, a Long-Acting Oxytocin Antagonist. Torsten M Reinheimer,[*1] Gerald Gimpl.[2] *[1]Non-Clinical Development, Ferring Pharmaceuticals A/S, Copenhagen S, Denmark; [2]Biochemistry, Johannes Gutenberg University, Mainz, Germany.*
Introduction: Barusiban is a novel, selective, oxytocin (OXT)-antagonistic peptidomimetic in clinical development for treatment of preterm labor. It offers the advantages of high potency and long duration of action in comparison to the tocolytic atosiban (TRACTOCILE), a registered mixed vasopressin V1a/OXT antagonist. Goal of the present study was to elucidate the biochemical mechanisms responsible for barusiban's long effectiveness and to confirm the reversibility of binding to the OXT receptor.
Methods: In the present study, HEK (Human Embryonic Kidney) and CHO (Chinese Hamster Ovary) cells expressing OXT receptors were employed. Association and dissociation constants were determined from membrane preparations and cell cultures by radioactive binding assays. Receptor internalization was investigated with tritiated antagonists following acid-wash procedures. OXT receptor functionality was measured by fura-2 labeled calcium increase. Receptor trafficking was monitored directly in cells expressing green fluorescent protein-tagged OXT receptors. OXT receptor desensitization was investigated with yellow fluorescent protein-labeled *beta*-arrestin.
Results: Barusiban and atosiban associated similarly to the OXT receptor but barusiban dissociated at an about 10-fold lower rate (half-life *ca.* 14 *versus* 1.4 min, respectively). The agonists OXT and carbetocin (DURATOCIN, PABAL) did induce receptor internalization. The OXT antagonists themselves did not lead to OXT receptor internalization. However, barusiban prevented OXT-induced receptor internalization for more than 24 hours. A fraction of about

20 percent of both OXT antagonists did internalize into the cells. Barusiban was about 30 times more potent than atosiban in preventing the OXT-induced intracellular calcium increase. None of the OXT antagonists was able to induce *beta*-arrestin translocation to the OXT receptor.
Conclusions: Both OXT receptor antagonists demonstrated competitive behavior and full binding reversibility. However, barusiban dissociated about 10 times slower from the receptors than atosiban. The antagonists prevented OXT receptor internalization and desensitization. About 20 percent of the antagonists penetrated into the cells. Barusiban in comparison to atosiban prevented the OXT-induced intracellular calcium response with an about 30 times higher antagonistic potency.

318

Pre-B Cell Colony Enhancing Factor (PBEF): Serum Concentrations in Pregnancy and Labor. Claire E Wright,[1] Jackie Shimoda,[2] Mark Hiraoka,[2] Peter Bryant-Greenwood,[3] Gillian Bryant-Greenwood.[*1] *[1]Pacfic Biosciences Research Center, University of Hawaii, Honolulu, HI, USA; [2]Obstetrics and Gynecology, John A. Burns School of Medicine, Honolulu, HI, USA; [3]Pathology, John A. Burns School of Medicine, Honolulu, HI, USA.*
Objective: To measure serum PBEF concentrations during pregnancy to gain insights into its roles in gestation.
Introduction: PBEF is a highly conserved pro-inflammatory cytokine with poorly understood biological activities. We have previously shown its up-regulation by NF-κB and AP-1 and by stretching. It acts as an insulin mimetic, interacting with the insulin receptor (Fukuhara et al. *Science* 2005: 307;426-430) and elevated levels of have been linked with obesity, (Haider et al. J Clin Endo Metab. 2006: 91;1578), type 2 (Chen et al. J Clin Endo Metab. 2006: 91;295) and gestational diabetes (Krzyzanowaska et al. *Clin Sci* 2006: 110;605). Therefore serum PBEF concentrations were measured in patients in labor with or without infection and with pregnancy complications.
Methods: Serum samples (205) were collected from women during pregnancy together with clinical data including; gestational age, medications, ethnicity and recognized complications. Patients were grouped according to labor status; no labor (107), early labor (33), term labor (47) and pre-term labor (18). Samples were centrifuged, aliquoted and stored at -80°C until use. The concentrations of PBEF were determined by EIA (Phoenix Pharmaceuticals) in accordance to the manufacturer's instructions.
Results: The concentrations of PBEF were between 5-100ng/ml, similar to values previously reported. Overall, there was no correlation between serum PBEF concentration and gestational age. The levels for non-laboring (non-infected) patients (n=46), showed an increase with gestational diabetes (n=15, p=0.051) and hypertension (n=7, p=0.0316). There was also an increase with term labor (n=28, p=0.036) compared to no labor (n=73), when infection was not taken into account. In patients with a range of clinical and histologically confirmed infection, PBEF was significantly increased at pre-term (n=6, p=0.039) and term labor (n=14, p=0.0165), compared those not in labor (n=29).
Conclusions: These results support a role for PBEF in labor as well as in infection-driven term and pre-term labor and suggests that PBEF is important in gestational diabetes and hypertension.
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319

The Psychophysiological Effects of Hydrotherapy on Anxiety and Pain in Human Labor. Rebecca D Benfield,[1] Tibor Hortobagyi,[2] Charles Tanner,[2] Edward Newton.[*3] *[1]ObGyn and SON, East Carolina University, Greenville, NC, USA; [2]Exercise and Sports Science, East Carolina University, Greenville, NC, USA; [3]ObGyn, ECU-Brody School of Medicine, Greenville, NC, USA.*
Objective: To examine and correlate the effects of hydrotherapy in laboring women on the anxiety, pain, stress hormones, oxytocin, and uterine contractility.
Methods: 11 healthy, term women in spontaneous active labor (cervical dilatation -range 3-6cm) were immersed to the xiphoid in 37°C water for one hour. Blood samples were drawn under dry baseline conditions and repeated at 15 and 45 min of hydrotherapy. Plasma epinephrine, norepinephrine, cortisol, oxytocin, vasopressin, and plasma volume % (PVS%) shift were analyzed. Visual analogue scales for anxiety and pain were administered before each blood draw. Telemetric monitoring of fetal heart rate and uterine contractions was used.
Results: Labor duration was normal (range-126-1294 min). Hydrotherapy was associated with a decrease in anxiety at 15 min ($p<.02$). Pain was decreased more for women with high baseline levels than women with low baseline levels after 15 ($p=.01$) and 45 min ($p=.04$) of bathing. Hydrotherapy was associated with a decrease in oxytocin at 15 min ($p=.02$) and 45 min ($p=.05$, trend). Contraction frequency decreased significantly ($p = .04$). Epinephrine and norepinephrine did not change significantly. Cortisol decreased twice as much after 15 min from baseline for the high baseline pain group (cortisol mean decrease = 6.2 mircograms/dL) compared to the low baseline pain group (cortisol mean decrease = 3.1 micrograms/dL). Plasma vasopressin decreased significantly after 15 min ($p=.02$) and after 45 min ($p=.02$) of hydrotherapy. All women had a positive PVS%, at 15 min the median PVS% was + 4.1% and at 45 min PVS% was +4.1%. PVS% at 15 min of hydrotherapy was positively correlated with contraction duration ($r = .74$, $p= .04$).
Conclusions: Hydrotherapy decreased anxiety and pain.The fall in stress hormones during hydrotherapy tentatively documents the reduction in pain and anxiety. Unexpectedly, a fall in oxytocin levels and a decrease in uterine contraction frequency were observed. The results may demonstrate two physiologic roles of oxytocin: it's traditional role in uterine contractility and a novel role as an anti-stress hormone.
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320

Parenchymal Collagen Content and Lung Maturation after Maternal Betamethasone Administration in a Fetal Rabbit Model. Xenia I Roubliova,[1] Lisbeth Vercruysse,[2] Pascal Vaast,[3] Paul J Lewi,[1,6] Jacques Jani,[1] Hui Qi Lu,[1] Dick Tibboel,[4] Eric K Verbeken,[5] Jan A Deprest.[*1,6] *[1]Center for Surgical Technologies, Catholic University of Leuven, Leuven, Belgium; [2]Laboratory of Experimental Gynaecology, University Hospitals, Leuven, Belgium; [3]CHU Jeanne de Flandre, Lille, France; [4]Department of Pediatric Surgery, Sophia Children's Hospital, Rotterdam, Netherlands; [5]Department of Pathology, University Hospitals, Leuven, Belgium; [6]Department of Obstetrics and Gynaecology, University Hospitals, Leuven, Belgium.*
Objective: To assess the effect of different doses maternal betamethasone (BM) on lung parenchymal maturation in the fetal rabbit model.
Study design: At d25-26 GA 8 does (58 fetuses) were injected with BM=0.05mg/kg/d, 9 does (45 fetuses) with BM=0.1mg/kg/d, and 8 does (56 fetuses) with normal saline. Does were sacrificed daily from d27 till term. Fetal lungs were formalin fixed for morphometry. % collagen was counted using purpose designed computer software. ANOVA (Tukey's test) was used($p<.05$: saline vs either *BM=0.05 or °BM=0.1).
Results: BM=0.05 increased alveolar size at d27. Both doses decreased collagen content in the lung parenchyma at d27 and 28. BM increased alveolar epithelial cell proliferation, reduced apoptosis and density of SP-B expressing cells.
Conclusion: Antenatal BM has a maturational effect on the fetal lung parenchyma.

Group	d27	d28	d29	d30	d31
Saline					
Lm, µm	80±4.3	74.3±4.8	70±3.3	67±7.4	74±6.8
Lmw, µm	43±3.1	31±3	30±4.6	34±5.8	26±6.3
Collagen content, %	11±1.5	12±2.2	9.3±2	8.1±3.5	8.2±2
SP-B+ cell density	1095±217	954±156	1039±221	1265±319	1335±372
PCNA+ cell density	1515±423	1301±492	1789±156	1489±433	1145±173
a-Caspase-3 cell density	2534±512	2047±667	3242±455	2754±465	2358±457
BM=0.05 mg/kg/d					
Lm, µm	*89±3.8	*85±5.5	74±4.2	69±8.7	72±5.4
Lmw, µm	49±5.2	34±4.2	37±7.3	34±6.8	35±9
Collagen content, %	6.5±1.2	*5.4±0.8	7.3±1.7	6.8±2.6	7.5±3.5
SP-B+ cell density	1317±163	1439±319	*1794±245	1532±195	1403±411
PCNA+ cell density	2468±725	*2653±341	2678±715	2045±314	1532±453
a-Caspase-3 cell density	*1152±486	1670±214	1859±683	1756±806	1935±514
BM=0.1 mg/kg/d					
Lm, µm	°72±3.4	73±5.1	70±7.1	73±9.2	66±5.4
Lmw, µm	°32±4.2	37±3.6	30±5.6	38±6.9	30±9.2
Collagen content, %	°3.9±1	°4.5±1.4	6.2±2	5.1±2	6.7±4.7
SP-B+ cell density	°1876±116	°1562±276	°1682±136	1620±342	1587±278
PCNA+ cell density	°3725±627	°2586±633	2488±835	2239±287	1856±522
a-Caspase-3 cell density	°935±443	°834±498	°1008±561	°1375±549	1469±716

FRIDAY

321

Expression of 5αReductase Enzymes in Uterine Tissues at Term and Following Labor. Della M Yates,[1] Hannah K Palliser,[1] David W Walker,[2] Jonathan J Hirst.[1] (SPON: Tamas Zakar). *[1]Mothers & Babies Research Centre, School of Biomedical Sciences, University of Newcastle, Newcastle, NSW, Australia; [2]Physiology, Monash University, Clayton, VIC, Australia.*

Objective: 5α-Reductases are key enzymes controlling the production of $GABA_A$ active steroids such as allopregnanolone. These steroids may have a role in protecting the fetal brain against excitotoxic injury. In addition, 5α-reduced steroids may have a role in influencing uterine excitability. 5α-reductase activity by the placenta may have a major role in the production of neuroprotective steroids in the maternal and fetal circulation as well as the fetal brain. The aims of this study were to compare the levels of 5α-reductase type 1 and 2 (5αR1 and 2) expression in the placentas of women at term and after labor in order to elucidate the role of the placenta in allopregnanolone synthesis.

Methods: Proteins were extracted from human placenta samples collected from term caesarean sections before labor (n=4) and following spontaneous labor (n=4) for immunoblotting. Densitometric analysis was used to compare the expression of 5αR1 and 2 protein relative to actin expression.

Results: 5αR2 expression was found to decrease significantly (72±10% decrease; $P<0.05$) in the placental samples obtained after labor compared to the term, non-labor samples. 5αR1 expression did not change significantly in association with labor.

Conclusions: The marked expression of 5αR1 and 2 suggests that both isoforms contribute to 5α-reductase activity in the placenta. The marked decline in 5αR2 expression may contribute to lowering allopregnanolone levels at labor and thereby place the fetus at risk of hypoxic-induced excitotoxic brain injury. The myometrium expresses $GABA_A$ receptors throughout gestation and at term (Erdo SL 1984 Biochem & Biophys Res Comms 125:18) and may regulate excitability. Since 5α-reduced steroids, including allopregnanolone, are potent $GABA_A$ receptor agonists, a fall in these $GABA_A$ active steroids may also contribute to the rise of myometrial activity at labor onset.

322

Identification and Functional Characterization of the Relaxin Receptor (LGR7) Splice Variants in the Human Decidua. Andras Kern, Aaron Amano, Gillian Bryant-Greenwood.* *Pacific Biosciences Research Center, University of Hawaii, Honolulu, HI, USA.*

Objective: To identify the splice variants of the relaxin receptor (LGR7) in the human decidua and show their functional significance.

Introduction: Relaxin is an autocrine/paracrine hormone in the human decidua. Its expression is increased in patients with preterm premature rupture of fetal membranes and its receptor (LGR7) is upregulated after preterm delivery. LGR7 is a leucine-rich repeat G-protein coupled receptor with several splice variants expressed in the human uterus and brain.

Methods: Decidua from twin pregnancies (n=4) before labor (30-37 wks) were collected and RNA isolated (Rneasy midi kit, Qiagen) and used for nested PCR with primers to the 5' and 3' untranslated regions of the cDNA. PCR products were cloned into pCR4Blunt-Topo vector (Invitrogen). For characterization, each was subcloned into pCR3.1 expression vector (Invitrogen) with or without hemagglutinin (HA)-tag. Expression in transfected HEK293 cells was detected by Western blotting with an antibody to the HA-tag. Glycosylation was shown by endoglycosidase treatment and subcellular localization confirmed by confocal microscopy using a fluorescent antibody to the HA-tag. Cell surface delivery of wild-type (WT)-LGR7 and splice variants was characterized by ELISA.

Results: The full-length LGR7 transcript and two unique splice variants (splice variant C and D) were identified in human decidua. Variant C was spliced between exon 12 and exon 18 and D between exon 5 and exon 16. Their expression varied markedly in the decidua from the four patients. Their expression in membrane fractions of transfected HEK293 cells was detected by Western blotting. Endoglycosidase treatment of the membrane fractions showed them to be highly glycosylated. The WT-LGR7 and variant D were both delivered to the cell surface, but C was not. When each splice variant was co-transfected together with the WT-LGR7, they reduced the cell surface delivery of WT-LGR7 to 36% and 28% respectively of the WT alone.

Conclusion: The full-length WT-LGR7 and two unique splice variants of the LGR7 are expressed in the human decidua. Both splice variants reduced the cell surface delivery of the full-length LGR7 exerting a dominant negative effect and are therefore functionally significant. Supported by NIH grant HD24314.

323

Role of Adipophilin in Basal and Cytokine-Induced Prostaglandin E_2 Synthesis from Lipid Droplets in Human Amnion Epithelial Cells. Kang Sun,[1,2] Frederick U Eruo,[1] Diane Brockman,[1] Leslie Myatt.*[1] *[1]Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA; [2]Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, China.*

Introduction Adipophilin, also known as adipocyte differentiation related protein (ADRP), is believed to be important for assembly of lipid droplets, thought to be intracellular foci for prostaglandin synthesis. However the role of adipophilin in this process has not been definitively characterized. The aim of this study was to explore the role of adipohilin in regulation of prostaglandin synthesis. **Methods and Results** We showed in this study intense immunostaining for adipophilin together with staining for the prostaglandin synthesizing enzymes cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase 2 (COX2) and microsomal prostaglandin E synthase (mPGES) on lipid droplets using dual immunofluorescent staining in both human amnion epithelial cells and amnion epithelium-derived WISH cells. Transfection of WISH cells with small interference RNA (siRNA) against adipophilin caused a significant reduction of adipophilin mRNA and protein expression as well as the number of lipid droplets coated with adipophilin, but not the number of lipid droplets *per se*. Consistently, there was a significant 3 fold reduction of basal prostaglandin E_2 (PGE_2) production in adipophilin siRNA treated cells vs control. In contrast Interleukin-1β (IL-1β, 10ng/ml) induced dramatic increases in COX2 expression and PGE_2 production in both adipophilin siRNA or negative control siRNA-treated cells and overcame the inhibitory effect of adipophilin siRNA treatment on PGE_2 production. **Conclusion** Adipophilin does not appear to be a crucial protein for formation of lipid droplets but may regulate basal PGE_2 synthesis at this site, i.e it has a functional rather than a structural role at the lipid droplet. The lack of inhibition of cytokine-induced PGE_2 synthesis by adipophilin siRNA suggests IL-1β may mediate PG synthesis via a different route.

324

Relaxin Stimulates Interleukin-8 Independently of Prostaglandin E2 in Human Lower Uterine Segment Fibroblasts. Rivka C Stone, Andrea Wojtczuk, Gerson Weiss,* Laura T Goldsmith.* *Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Newark, NJ, USA.*

Objective: Elevated circulating maternal relaxin concentrations are associated with preterm birth in singleton IVF pregnancies, whose incidence of prematurity is double that of naturally conceived singleton pregnancies. Relaxin's effects upon the primate cervix include reorganization of collagen and elastin fibrils and increased matrix metalloproteinase (MMP) activity. Prostaglandins and inflammatory cytokines play important roles in cervical ripening prior to delivery. Interleukin (IL)-8 acts to recruit neutrophils, additional sources of MMP activity, to the cervix at term. We tested the hypothesis that relaxin regulates cervical IL-8 production via stimulation of Prostaglandin (PG)E2 in human lower uterine segment fibroblasts.

Methods: Fibroblasts isolated from human lower uterine segment tissue taken at term pregnancy (LUSF) were incubated in the absence or presence of human relaxin, 1-100 ng/ml, for 72 hours. Media were removed and assessed for Il-8 and PGE2 concentrations by specific enzyme immunoassays.

Results: Il-8 and PGE2 were detectable in media from LUSF. At the lowest dose of relaxin, 1 ng/ml, no significant effect upon IL-8 was observed. However, at doses of 10 and 100 ng/ml, relaxin significantly stimulated IL-8 to mean levels of 211± 2 ($p=0.018$) and 253±12 ($p=0.004$) percent above control, untreated cells respectively (n= 3 experiments, each conducted in triplicate). In contrast, media PGE2 levels were not affected by relaxin.

Conclusions: Relaxin significantly stimulates IL-8 production by LUSF through a PGE2 independent pathway. This action of relaxin in the cervix may contribute to the cervical ripening component involved in preterm delivery.

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325

Differential Expression of Novel Phospholipase A_2 Enzymes in Human Gestational Tissue. Donna M Slater,[1,3] Elizabeth King,[2] Steven Thornton,[*3] Manu Vatish.[3] *[1]Pharmacology and Therapeutics, University of Calgary, Canada; [2]Department of Cell Biology & Anatomy, University of Calgary, Canada; [3]Warwick Medical School, United Kingdom.*

Introduction: The increased prostaglandin synthesis observed within the uterus at term may be mediated by increased expression of cyclo-oxygenase-2. However, increased COX activity is likely to require concomitant increases in the release of substrate arachidonic acid, catalysed by the action of one or more phospholipase A_2 (PLA_2) enzymes. Numerous distinct PLA_2 isozymes have been identified, differing in molecular size, cellular localisation and substrate specificity. Two classes; secretory ($sPLA_2$) and cytosolic phospholipases ($cPLA_2$) are further classified into groups I, II, III, V, X and IV, VI, VII, VIII respectively. In myometrium, there is a pregnancy and labour associated increase in the expression of $sPLA_2$-IIA, but this does not account for the overall increase in PLA_2 activity observed during pregnancy and labour.

Objectives: To identify the array of PLA_2 genes that are expressed in human gestational tissues and myometrial smooth muscle (MSM) cultures.

Methods: Amnion, chorio-decidua and placenta were collected from women who delivered at term, either before or after the onset of labour. Myometrial biopsies, from lower uterine segment and upper uterine segment (fundus), were taken from non-pregnant women at hysterectomy and women at caesarean section before or after labour onset. Samples were either snap frozen and stored at -80°C prior to RNA isolation or used immediately for culture of MSM cells. A search of the human genome mapping project and mammalian data-bases was performed to identify PLA_2 genomic sequences. Reverse transcription polymerase chain reaction (RT-PCR) primers were designed to identify individual PLA_2 genes, and splice variants where appropriate. RT-PCR products were analysed and verified by sequence analysis. Subsequently, to determine any tissue-specific expression patterns we utilized real-time RT-PCR.

Results: Expression of PLA_2-IIA, PLA_2-IV, PLA_2-V and PLA_2-VII was verified. We also identified the novel expression of PLA_2-IID (placenta>amnion, chorio-decidua), PLA_2-III (amnion epithelial cells) and PLA_2-X (all tissues).

Conclusion: The presence of novel PLA_2 genes in human gestational tissues, suggests the possibility that they may contribute to the release of arachidonic acid within these tissues.

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326

The Measurement of Maternal Plasma Urocortin for the Early Prediction of Preterm Delivery. Alberto Imperatore,[1] Pasquale Florio,[*1] Michela Torricelli,[1] Giulia Calonaci,[1] Elisa Faldini,[1] Daniela Dores,[1] Elisabeth A Linton,[2] Felice Petraglia.[*1] *[1]Chair of Obstetrics and Gynecology, Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy; [2]Nuffield Departement of Obstetrics and Gynecology, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom.*

Objective: Preterm birth remains a management problem despite major advances in our understanding of it. Urocortin is a 40 amino acids peptide expressed by gestational tissues, whose maternal levels correlate to the status of human myometrium, increasing at term and preterm delivery. The aim of this study was to evaluate whether in women with threatened preterm labor, maternal plasma urocortin concentrations differ between patients who deliver at term and those preterm and if its measurement might be useful in predicting preterm delivery in women with threatened preterm labor. **Methods:** 85 singleton pregnancies, that experienced threatened preterm labor, were evaluated. Maternal blood samples obtained at the time of admission were stored at -80°C. A specific RIA assay was performed to measure urocortin levels. **Results:** Thirty out of 85 patients delivered preterm. Patients who delivered preterm were subdivided into those that delivered less than or equal to 7 days and greater than 7 days between the time of admission (sampling) and parturition. Twenty-three out of 30 patients delivered before 7 days from the time of admission whilst the remaining delivered later. Maternal plasma urocortin concentrations were significantly higher in women who delivered preterm than in those who progressed to term. Urocortin concentrations in patients delivered preterm negatively correlated to the delivery time interval (in days) and were significantly higher in those who delivered before than after the 7 day interval. At the cutoff 113.93 pg/mL, urocortin achieved a sensitivity of 80% and a specificity of 100% as single marker for prediction of preterm labor. When urocortin levels were found above the thresholds defined by the ROC curve analysis, the probability of preterm delivery was as high as 100%, whilst the probability was 9.83% when found unaltered. **Conclusion:** maternal plasma urocortin levels are increased in patients with threatened preterm labor who delivered before 34 completed weeks of pregnancy and their measurement is a promising new biochemical marker to add significant prognostic information for predicting preterm delivery among women at risk.

327

Phorbol Ester Stimulates Corticotropin-Releasing Hormone Gene Promoter Activity through a cAMP Regulatory Element in Primary Placental Cells. Yue Hou,[1] Xiaolu Tang,[1] Richard C Nicholson,[1,2] Xin Ni.[1] (SPON: Tamas Zakar). *[1]Department of Physiology, Second Military Medical University, Shanghai, China; [2]Mothers and Babies Research Centre, John Hunter Hospital, Newcastle, NSW, Australia.*

Objective: Placental CRH plays a major role in the mechanisms controlling human pregnancy and parturition. During human pregnancy, biosynthesis and secretion of placental CRH increases exponentially with advancing gestation, and this increase is mirrored by exponential increases in CRH concentration in maternal plasma. Various endogenous factors have been shown to regulate the placental CRH gene expression. In this study we aim to determine the role of phorbol-12-myristate-13-acetate (PMA), a PKC activator, in the regulation of CRH promoter activity in primary cultures of placental cells.

Results: Using primary placental cell cultures transfected with CRH promoter-luciferase reporter plasmids, PMA simulated CRH gene promoter activity in a dose-dependent manner with significant ($P<0.01$) activity at 24 hours following treatment with 10-7 M to 10-5 M PMA. The use of progressive 5'-deletion analysis of the CRH promoter sequences shows that the region between –248 and –213bp, containing a consensus cAMP regulatory element (CRE), is essential for PMA responsiveness following exposure to 10-6 mol/L PMA for 24 hours. Specific mutation studies of the CRE, and the use of CRE sites linked to heterologous basal promoters, have determined that this site is essential for PMA actions. Furthermore, 17 beta-estradiol (10-7 mol/L) treatment of transfected primary placental cells resulted in the significant decrease ($P<0.01$) of both basal and PMA-stimulated (10-6 mol/L) CRH promoter activity when the CRE element was present but had no effect when the CRE was absent.

Conclusion: PMA stimulates CRH gene transcriptional activity through the CRE suggesting that cross-talk of PKC and PKA pathways may occur at the CRE in placental cells. Estrogen suppresses this PMA-induced activity, via mechanism(s) that require the CRE.

328

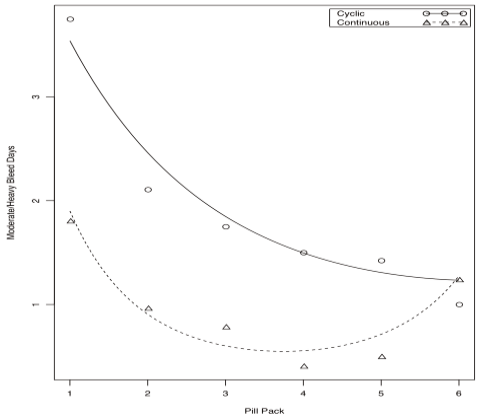
An RCT of Extended Cycle vs 28d Cyclic OCP Therapy on Endometrial and Ovarian Function. Jaimey M Pauli,[1] Richard J Zaino,[1] Carol G Gnatuk,[1] James S Kesner,[2] Juliana W Meadows,[2] Larry M Demers,[1] Allen R Kunselman,[1] William C Dodson,[*1] Richard S Legro.[*1] *[1]Ob/Gyn, Pathology, and HES, Penn State College of Medicine, Hershey, PA, USA; [2]Reproductive Endocrinology Laboratory, NIOSH, Cincinnati, OH, USA.*

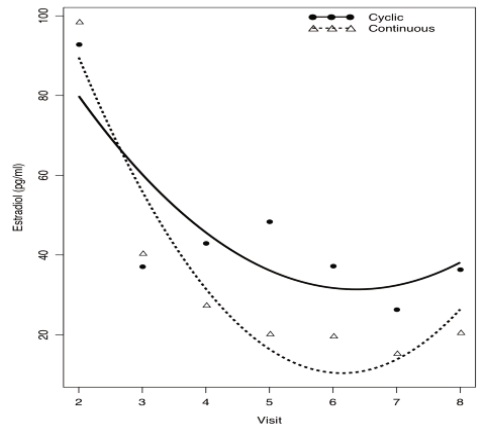
a) Hypothesis: 168d Extended Cycle(EC) suppression with oral EE 20 mcg/NETA 1 mg provides greater ovarian & endometrial suppression than 28d Traditional Cycle(28C) therapy with equal safety.

b) Methods: We performed a double blind RCT of these 2 regimens in 63 normally cycling women & followed bleeding, endometrial histology by biopsy, follicular development by serial ultrasound, and serum and urinary levels of sex steroids.

c) Results: The randomized groups were well matched at baseline. Generalized estimating equations using a log link were fit to bleeding patterns across visits, and there were no differences between groups due to a rebound increase in the EC arm by study end(Fig 1, P=0.25). Quadratic random coefficients models were fit to analyze continuous outcomes across multiple visits. There was greater suppression of serum E2 levels in the EC arm(P=0.02, Fig 2). Total ovarian volume (cm^3) was decreased more in the EC arm(-9.0 vs +0.8 for 28C, P=0.02), as did the mean diameter(mm) of the largest follicle(-2.3 vs +5.3, P=0.001). Area under the curve(AUC) for E1G (ng/mg Cr) from daily urines in a subset of 20 subjects was significantly less in the EC arm(AUC per day

median 9.1 vs 17.4, P=0.04). Ovulation by urinary PdG measures occurred in 5/10 subjects in 28C and 1/10 in EC. There were no differences in safety outcomes(histology, B/P, BMI, SHBG, G, I, or lipids) between groups.
d) Conclusions: This study provides consistent data of greater ovarian suppression with EC treatment and forms the basis for its use in mulitple disorders (i.e. endometriosis, hirsutism, acne). Supported by RO1HD433332(RSL).





329

Cerebral Glucose Metabolism in the Menstrual Cycle: Preliminary Study of Premenstrual Dysphoric Disorder. Andrea Rapkin,[*1] Steven M Berman,[2] Melinda Morgan,[1] Edythe D London.[2] *[1]Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [2]Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*
Objectives: To clarify the basis for symptoms of premenstrual dysphoric disorder (PMDD), by assessing brain function, indexed by relative regional activity measured with positron emission tomography (PET) and [F-18] fluorodeoxyglucose, in the follicular phase and the late luteal phases in women with PMDD and controls.
Methods: All subjects prospectively documented symptoms using the Daily Record of Severity of Problems and PMDD subjects fulfilled DSM 4 criteria for PMDD. Controls were asymptomatic. Subjects documented ovulation using urinary lutinizing hormone detection kits. Estradiol and progesterone concentrations and PET scans using [F-18] fluorodeoxyglucose were performed between days 6 to 9 and 12-14 days after ovulation. Comparisons between groups and phases were made using Statistical Parametric Mapping. Hormonal and mood data was evaluated using analysis of variance with repeated measures.
Results: 4 women with PMDD and 5 controls have completed the ongoing study. PMDD subjects, but not controls, experienced a significant increase in symptom number and severity in the luteal phase compared with the follicular. Regional Cerebral Glucose Metabolic Activity: Across all subjects, relative activity was lower in the late luteal phase (vs. follicular), primarily in the inferior aspect of the left superior temporal gyrus and the middle temporal gyrus. In contrast, relative activity was greater during the late luteal phase than in the follicular phase, primarily in the superior aspect of the right superior temporal gyrus. The left hemisphere effect seen in the entire sample was modified by a significant interaction with subject group, whereas this was not true of the right hemisphere effect. The interaction shown by the contrast "Late Luteal < Follicular; CONTROL > PMDD," was significant, extending from the left superior temporal gyrus, into the middle temporal gyrus and posterior insula. Conclusions: The results in the controls are consistent with left lateralization of affective valence in the luteal phase. The left hemisphere is dominant for positive emotions while right is associated with negative emotions. PMDD subjects fail to demonstrate left lateralization of functional brain anatomy in the luteal phase to the same degree as control subjects.

330

Intrauterine Release of Progesterone Antagonist ZK230211 Is Feasible and Results in Unique Endometrial Effects. Oskari Heikinheimo,[*1] Susheel Vani,[2] Olli Carpen,[3] Paivi Harkki,[1] Annamaija Tapper,[1] Eeva-Marja Rutanen,[1] Hilary Critchley.[*2] *[1]Department of Obstetrics and Gynecology, University of Helsinki, Helsinki, Finland; [2]Division of Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, United Kingdom; [3]Department of Pathology, University of Turku, Turku, Finland.*
Objective: Continuous administration of progesterone antagonists (PA) results in endometrial suppression and amenorrhea. We studied the endometrial effects of intrauterine release of PA ZK230211 (ZK-IUS) in comparison to those seen with use of a levonorgestrel releasing intrauterine system (LNG-IUS) among women scheduled for hysterectomy because of heavy or painful menstruation.
Patients and methods: 42 women were randomly fitted with IUSs releasing ZK230211 at rate 1, 4 or 8 μg/24h, or 20μg/24h of LNG 4 to 8 weeks before hysterectomy. Uterine bleeding and endometrial morphology were evaluated. Immunohistochemistry (IHC) was performed for proliferation markers (Ki67 and PH3), estrogen receptors (ER α and β), progesterone receptor (PR), and insulin like growth factor binding protein-1 (IGFBP-1).
Results: Bleeding profile was not altered by the use of ZK-IUSs. ZK230211 was measurable in all endometrial specimens. Endometrium was categorized as partly suppressed in 9 to 30% following the use of the ZK-IUSs whereas suppressed endometrium was seen in 67% following the use of the LNG-IUS. Morphological signs of estrogen effect were evident in 60-82% vs. 11% of the endometria following the use ZK-IUSs vs. LNG-IUS, respectively ($p<0.05$). However, IHC for Ki67 or PH3 demonstrated negligible proliferative activity in endometria exposed to either ZK- or LNG-IUS. The use of ZK-IUS resulted in high level of PR expression in the endometrial surface epithelium and stroma. PR was not detectable in surface epithelium, and expressed only at low levels in the stroma following the use of LNG-IUS. Expression of ERs did not differ between the two groups. IGFBP-1 was detectable in 89% of endometria exposed to LNG whereas it was absent in all specimens exposed to ZK230211.
Conclusions: Intrauterine release of PA ZK230211 is feasible. Expression of the proliferation markers Ki67 and PH3 was negligible following the use of both ZK- or LNG-IUS. Lack of decidualization marker IGFBP-1 and high level of PR immunostaining following the use of ZK-IUS reflect the PA effects of ZK230211. Clinical utility of the ZK-IUS warrants further evaluation.

331

Pathologic Features Following Failed Endometrial Ablation. Albert L Hsu, Carrie Bell, Ronald Burkman, Daniel Grow. (SPON: Charles C Coddington). *Obstetrics and Gynecology, Baystate Medical Center, Springfield, MA, USA.*
OBJECTIVE: to characterize the pathologic findings in patients who have had a hysterectomy after endometrial ablation
METHODS: retrospective chart review of patients identified by CPT/ICD-9 billing codes as having hysterectomy subsequent to endometrial ablation.
RESULTS: Between 2000 and 2005, 1514 endometrial ablations were performed at Baystate Medical Center. In that same time period, 227 hysterectomies (15%) were performed after a prior endometrial ablation. Indications for hysterectomy included bleeding as well as prolapse, ovarian pathology, pain, and endometriosis.
Review of specimens from 53 subjects undergoing hysterectomy between January 2004 to December 2005 revealed the pathologic findings noted in Table 1. Adenomyosis and fibroids correlated with larger uterine weights. Among three patients who had a hysterectomy for endometrial adenocarcinoma, two had benign findings on endometrial biopsy prior to ablation and one had "quantity not sufficient for diagnosis" on biopsy prior to ablation.
CONCLUSIONS:
Global endometrial ablation devices like ThermaChoice and Novasure are quick, safe, cost-effective, and transitioning to an office-based procedure. As patients increasingly request endometrial ablation in lieu of hysterectomy, preoperative imaging remains an important step to exclude large myomata or polyps, which

may be better treated with hysteroscopic resection or hysterectomy. Our findings suggest a preoperative sonohysterogram or MRI may help predict ablation failure by ruling out adenomyosis or submucous fibroids.
To our knowledge, this is the largest study of hysterectomies after endometrial ablation. Pathologic characterization of uterine specimens will continue for all hysterectomy cases from 2000-2005. Our findings underscore the importance of obtaining a repeat endometrial biopsy when symptomatic uterine bleeding recurs after endometrial ablation. Finally, in patients with prolapse or endometriosis, permanent relief offered by definitive management with hysterectomy may be superior to an endometrial ablation.

Pathology Noted After Hysterectomy

	Patients	Adenomyosis	Fibroids	Endometriosis	Cancer
Novasure	34	18 (53%)	15 (44%)	8 (24%)	3 (9%)
Balloon	6	1 (17%)	3 (50%)	0	0
Rollerball	13	6 (46%)	6 (46%)	1 (8%)	0
Totals	53	25 (47%)	24 (45%)	9 (17%)	3 (6%)

332

Safety and Feasibility of Laparoscopy in the Management of Tubal Ectopic Pregnancy in Obese Women. Mazen E Abdallah, Rahi Victory, Dana R Ambler, Jay M Berman, Elizabeth E Puscheck, Michael Diamond.* *OBGYN, Wayne State University, Detroit, MI.*
Objective: To study the safety and feasibility of laparoscopic management of tubal ectopic pregnancy (EP) in obese vs. lean women.
Design: Retrospective chart review study between 1/2000-2/2005 at Hutzel Women's Hospital, Detroit, Michigan.
Methods: Cases who had laparoscopic management of EP were identified. Abstracted data included demographic variables, gestational age, pain and bleeding level, vital signs and laboratory values (hemoglobin, hematocrit, βHCG). Weight, height, BMI, ultrasound (U/S) findings (size, fetal heart beat and cul de sac fluid), failed methotraxate use and history of abdominal surgery or ectopic pregnancy were recorded. Records were reviewed for physician subspecialty (MFM, REI, gynecologist), American Society of Anesthesiologists (ASA) score, operative procedure (laparoscopy or conversion to laparotomy), intraoperative findings (hemoperitonium, ruptured EP or adhesions), estimated blood loss (EBL), procedure duration, complications, and hospital stay. Cases were divided into 2 groups depending on BMI, lean (BMI < 30) and obese (BMI ≥ 30); each group was divided into 2 subgroups whether or not converted to laparotomy. Statistical analysis was performed using chi square and student *t* test. Results were significant when $p<0.05$.
Results: 368 cases of EP had laparoscopy and 67 (18.2%) cases were converted to laparotomy. 37.6% of the cases were obese with mean BMI 35.9 (+/- 5.4). Obese women did not differ from lean women in age, gravidity, parity, gestational age, previous abdominal surgery or EP, pain or bleeding level, laboratory values, or U/S findings. However, there was a difference in ASA score (10.2% of obese vs. 4.2% of lean had high ASA score, $p=0.006$) and failed methotraxate use (7.7% in obese vs. 15.3% in lean, $p=0.038$). There was no difference in the conversion rate between the obese and the lean groups (20 vs18%, $p=0.6$), nor did the groups differ in physician subspecialty, procedure duration, EBL, intraoperative findings, reason for conversion, incidence of intraoperative or postoperative complications and hospital stay.
Conclusion: Obesity did not increase the likelihood of laparoscopic failure in EP treatment. Laparoscopy did not seem to increase morbidity in obese women undergoing surgical EP treatment when compared to lean women. There was no increase in conversion rate, intraoperative or postoperative complications, EBL, procedure duration or hospital stay.

333

Is Sono-Hysterography Comparable to Hysterosalpingography in Evaluating Tubal Patency? Afarin Y Greiger, William Stohsnitter, Sasmira Lalwani. (SPON: David Chelmow). *Ob/Gyn, Tufts New England Medical Center, Boston, MA, USA.*
Objective:
To evaluate Sonohysterogram(SHG) as a screening test to assess tubal patency in infertile women. The presence of fluid in the cul-de-sac after SHG was used as a marker of unilateral tubal patency, and compared to hysterosalpingogram(HSG) in diagnosing unilateral tubal patency.
Material and Method:
The study population included infertile women, attending our Reproductive Endocrine department between June 2004-June 2006 who required both HSG and SHG as a part of their workup.
The adnexa and culdesac were initially evaluated using transvaginal ultrasound, prior to performance of SHG. Accumulation of fluid in the culdesac on saline SHG was considered as criteria for unilateral tubal patency. Results were compared with HSG findings.
Results:
30 patients participated in the study. 3 patients were excluded due to technical difficulties.
24 patients had bilateral tubal patency, 3 had unilateral patency and no patient had bilateral occlusion on HSG.
22 patients (81%) had fluid in the culdesac after SHG indicating at least unilateral patency. 5 patients (19%) had no fluid in the culdesac. All of these 5 patients had bilateral tubal patency on HSG. Hydrosalpinx was diagnosed on SHG in 1 patient and confirmed on HSG.
Concordance and sensitivity for unilateral patency was 81.5%. Specificity, PPV and NPV for patency could not be measured.
Specificity for bilateral tubal blockage was 81.5%.
Discussion:
Saline SHG was evaluated as an initial screening test for determining unilateral tubal patency, based on the premise that unilateral patency on HSG is associated with normal fecundity rate ratio. SHG is also more sensitive than HSG in evaluating uterine cavity.
Sensitivity for tubal patency in our study was 81.5% which is Similar to results in other studies comparing SHG and HSG in evaluating tubal patency.
Our study is different from other studies in that only presence of fluid in the culdesac after SHG was used as a criteria for determining unilateral tubal patency. Other studies have used various dyes and more complicated techniques to evaluate tubal patency.
Saline SHG is a very simple procedure that may indeed be a useful primary screening test specially in low risk infertile population that can evaluate uterus and tubes simultaneously. Patients with abnormal results on SHG should undergo HSG as secondary test.

334

The Impact of the 80 Hour Workweek Restriction on Resident Participation in Research Projects and Journal Clubs. Eve C Feinberg,[1] Whitney B You,[2] Alan H DeCherney.*[1] *[1]Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; [2]Obstetrics and Gynecology, Uniformed Services Residency in Obstetrics and Gynecology at the Uniformed Services University of the Health Sciences, Bethesda, MD, USA.*
Objective:
With the advent of the 80 hour workweek, fewer hours are available for scholarly activities such as resident research and journal clubs. While these are undoubtedly important for professional growth, the increasing time constraints placed on Residents might limit such activities. Participation in both resident research and journal clubs are currently mandated by the RRC, however, the degree to which residency programs are compliant varies greatly between institutions. The purpose of this study was to evaluate the level of participation in resident research projects and journal clubs among residency programs in the U.S. and to highlight the perceived impact of participation on future career choices.
Methods:
A 30 item questionnaire was sent to all members of the Society for Gynecologic Investigation. Residency programs that did not have Society members were contacted individually though an emailed questionnaire to the Program Director or Chairman. Questions pertaining to resident research and journal club were both quantitative and descriptive. For research, quantitative questions such as percent residents involved in research, time allotment for research, numbers of publications and presentations, and amount and sources of funding were asked. Descriptive questions such as type of research (basic science, chart review, case report), mentorship and perceived impact of research on future fellowship training were also asked. Journal club inquiries were geared towards attendance, participation, type of material presented and whether articles presented impacted practice. Individuals were asked what the RRC requirement for journal club entails.
Results: The respondents were given 6 weeks to return the survey.
Conclusions: Despite the 80 hour workweek restrictions, most residency programs were actively involved in resident research and journal clubs. There was, however, a large variation between programs with regard to these scholarly activities.

335

Comparison between Laparoscopic and Vaginal Hysterectomy for Benign Pathologies in Patients with a Uterine Volume Less Than 300 cc. Prospective Randomized Study. Massimo Candiani, Stefano Izzo, Enrico Betto, Jennifer Riparini, Nicola Berlanda. (SPON: Anna Maria Marconi). *Obstetric and Gynecology, San Paolo Hospital, Milan, Italy.*

STUDY OBJECTIVE: to compare laparoscopic and vaginal hysterectomy.

METHODS: Prospective, randomized, controlled comparison between vaginal (VH) and laparoscopic (LH) hysterectomy among 50 consecutive patients with a uterine volume ≤ 300 cc and without uterine prolapse. Follow up was 12 months for all patients.

RESULTS: the characteristics of the patients were comparable for mean age (VH:51; LH:48; p=0.364), mean uterus volume (VH:166; LH: 173; p=0.729), nulliparity (p=0.087), previous pelvic surgery (0.569) and indications to surgery (myomas (VH: 73%, LH: 50%), adenomyosis (VH: 9%; LH: 22%), ovarian cysts (VH: 0%; LH:10%), endometrial iperplasia (VH: 18%; LH: 18%)). The two groups were significantly different for BMI (VH:28; LH:24; p=0.013), for mean operative time (VH: 81 (40-150) minutes, LH: 99 (60-180) minutes; (p=0.033) and for mean blood loss (LH: 83 ml, VH:178ml; p=0.004). In only 73% of vaginal arm the bilateral adnexectomy was performed when preoperatively planned; this happened in 100% of laparoscopic arm (p=0.005).

Postoperative pain in the first day (VAS: VH: 5,17; LH: 2.74; p=0.023) and the number of days of analgesic request was higher in vaginal group (LH: 0.96; VH: 1.65; p=0.017). We described a major complicance (trombosis at day 6) in one patient of vaginal group (p=0.173) that was treated with eparin and had a spontaneous resolution. At six months clinical evaluation we found an increment, although not statistically significative, of pelvic floor defects in vaginal arm (p=0,112). This evidence was not confirmed at 12 months follow-up.

CONCLUSION: laparoscopic hysterectomy is associated with an increased of operative time (p=0.033) but also to a reduction of blood loss (p=0.004) if compared with vaginal hysterectomy. Adnexectomy may be difficult or dangerous during vaginal hysterectomy; when planned it was performed in 73% of patients only in this group vs 100% in laparoscopic one. Even if not significantly laparoscopic hysterectomy is associated with an important reduction of postoperative pain and analgesic request compared to vaginal hysterectomy. The techniques are comparable in terms of patient satisfaction, urinary symptoms and gynaecological objectivity at 12 months follow-up.

336

Vaginal Nonoxynol-9 Induces Endometrial Apoptosis: Implications for Risk of HIV Infection. Aimin Li,[1] Megan A Economidis,[1] Juan C Felix,[1,2] Wangrong Yang,[1] John K Jain.[*1] *[1]Dept. of Ob/Gyn, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; [2]Dept. of Pathology, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA.*

Objectives:

Previously, we have demonstrated that N-9 induced apoptosis in a human endometrial explant model. The present study was designed to evaluate the effect of in vivo vaginal application of N-9 to human endometrium.

Methods:

Twenty-five regularly-menstruating women were randomized to receive 1 or 3 applications of N-9 in the proliferative (D9) or secretory (D22) phase of the menstrual cycle. Endometrial biopsies were obtained before and after the N-9 treatment. Apoptosis was determined by TUNEL and cleaved caspase-3 immunostaining. Endometrial proliferation rate and mucin content was assessed immunohistochemically with Ki-67 and MUC-1, respectively. Expression of apoptosis-related genes, Bcl-2, Bax, Fas receptor (Fas) and Fas ligand (Fas L) was quantified using real- time PCR.

Results:

N-9 exposure led to a significant increase of TUNEL-positive cells in human endometrium relative to pre-N-9 endometrium (*n*=24). A similar trend was noted in both D9 (*n*=11) and D22 (*n*=13) groups. The cleaved Cas-3-positive cells were also increased significantly after one application of N-9 in D9 group. Ki-67-possitive cells in endometrial stroma was significantly reduced after N-9 exposure. A consistent low expression of Ki-67-positive cells was noted in the stroma of each group studied. No such significant changes of MUC-1 expression were detected in human endometrium after N-9 exposure. Real-time PCR analysis (*n*=12) revealed that all 12 women analyzed show a similar trend of increased *Bax* expression after N-9 exposure while 8, 6 and 9 out of 12 women showed increased *Bcl-2, Fas* and *FasL* expression, respectively.

Conclusions:

These data suggested that vaginal N-9 could induce endometrial apoptosis implicating the upper reproductive tract as a portal of entry for HIV infection. We suggest that the upper reproductive tract should be tested when developing future contraceptive microbicides.

337

Decreased Endometrial HOXA10 Expression Associated with Copper Intrauterine-Device Use. Amy M Tetrault, Susan M Richman, Xiaolan Fei, Hugh S Taylor.[*] *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objectives: HOXA10 is a transcription factor that is expressed in human endometrium. The expression of HOXA10 is essential for adult endometrial receptivity to achieve blastocyst implantation. HOXA10 expression is regulated by sex steroid hormones and markedly increases during the midsecretory phase, corresponding to the time of implantation. Altered expression of HOXA10 has been associated with decreased implantation rates in women. Here we characterized human endometrial HOXA10 expression in patients using copper intrauterine devices (IUD).

Methods: Immunohistochemical analysis was used to determine endometrial HOXA10 expression. At the time of IUD removal, endometrial tissue samples were obtained from 17 women who were using copper containing Paraguard T380A. Control endometrial biopsy samples were collected from 10 normal cycling women who were matched for menstrual cycle stage, and who were not using an IUD or hormonal contraceptive method. Immunohistochemistry was performed using a polyclonal antibody to identify HOXA10 expression in human endometrial tissue. Analysis of HOXA10 expression was quantified using the H score method and Mann-Whitney Rank Sum Test.

Results: Endometrial HOXA10 expression, which is localized to the nucleus, was markedly decreased in women using IUD contraceptives when compared to the control group. The mean H score for endometrial stromal expression of HOXA10 in women using Paraguard IUD was 0.52 compared to 2.7 in the control endometrial biopsies (p=0.04). The glandular expression of HOXA10 was absent in the endometrium exposed to the IUD.

Conclusions: Decreased endometrial HOXA10 expression is apparent in women who use a copper IUD. The expression of HOXA10 is essential for endometrial receptivity. A novel mechanism of Copper IUD action is by regulation of genes required for endometrial receptivity. The dramatic decrease of endometrial HOXA10 in response to IUD usage may contribute to the enhancement of contraceptive effectiveness.

338

Low Dose Mifepristone Suppresses Breakthrough Bleeding Induced by Levonorgestrel Intrauterine Devices in Rhesus Macaques. Ov D Slayden,[*1] Hilary Critchley,[*2] Rebecca Carroll,[1] Yun-Yen Tsong,[3] Regine Citruk-Ware,[3] Robert Brenner.[*1] *[1]Div. of Reprod. Sciences, Oregon National Primate Res. Center, Beaverton, OR, USA; [2]Centre for Reprod. Biology, University of Edinburgh, Edinburgh, United Kingdom; [3]Center for Develop. Res, Population Council, New York, NY, USA.*

Breakthrough bleeding (BTB) remains a drawback to the use of levonorgestrel-releasing intrauterine systems (LNG-IUS) for contraception. **Objective:** Because mifepristone can suppress the BTB associated with contraceptive use of systemic progestins (Steroids 68:1115-9; 2003; Hum Reprod 21:295-302; 2006) we determined whether BTB induced by an LNG-IUS could also be suppressed by mifepristone in a nonhuman primate model. **Methods:** Adult rhesus macaques (n=12) were ovariectomized and treated with subcutaneous Silastic capsules of estradiol and progesterone to induce a 28-day menstrual cycle (Hum. Reprod.8:1562-74; 2001). On cycle day 7 a miniature LNG-IUS was inserted. Vaginal swabs to assess bleeding were performed daily for 6 cycles. In the 3rd cycle, animals that displayed BTB (n=4) were treated with mifepristone (1 mg/kg/day) for 3 days. Uteri were collected at the end of the 6th cycle. **Results:** In all animals**,** the LNG-IUS decidualized the endometrium and prevented cyclic menstruation. In the four mifepristone treated animals, irregular BTB occurred at a rate of 9.8±2.4 bleeding days/ cycle before treatment and a rate of 0.31±0.11 bleeding days /cycle after treatment (P<0.05). Mifepristone initially triggered 3-4 days of typical menstruation, followed by a mean interval of 62±19 bleeding-free days after which BTB resumed. Immunocytochemistry showed that typical markers of decidualization (TIMP-3, IGF-BP) were induced by the LNG-IUS in the macaque endometrium. **Conclusions:** In rhesus macaques, as in women, insertion of an LNG-IUS decidualized the endometrium, suppressed cyclic menstruation and induced BTB in 1/3 of the individuals. In the latter,

short term treatment with mifepristone suppressed BTB for over 2 months. How this brief treatment induced such sustained effects remains to be clarified, but the data suggest that intermittent, low dose therapy with progesterone receptor antagonists can suppress BTB induced by the LNG-IUS. Supported by HD 43209 and RR 00163.

339

Effects of Depo Medoxyprogesterone Acetate on Glucose Tolerance in a Patient with Generalized Lipodystrophy: A Case Report and Review of the Literature. Belinda J Yauger,[1] Phillip Gorden,[2] Jean Park,[2] Elaine Cochran,[2] Pamela Stratton.[*1] *[1]Reproductive Biology and Medicine Branch, NICHD, NIH, Bethesda, MD, USA; [2]Clinical Endocrinology Branch, NIDDK, NIH, Bethesda, MD, USA.*

Hypothesis: Lipodystrophy, an uncommon congenital or acquired condition, is characterized by loss of adipose tissue, low leptin levels, and increased risk for insulin resistance often resulting in diabetes and dyslipidemia. Depo medoxyprogesterone acetate (DMPA) has been associated with worsened glucose tolerance especially in diabetics. DMPA may worsen baseline insulin resistance in such a population.

Methods: An 18 year old female with congenital generalized lipodystrophy had significant improvement in glycemic control after experimental 3.5 mg/d leptin therapy, requiring 1000mg/d of Metformin but no insulin. After an injection of DMPA in June 2006, she was admitted with severe hyperglycemia, hypertriglyceridemia, and elevated HgbA1c.

Results: Glucose control and triglyceride levels eventually returned to near-normal with extremely high doses of insulin (up to 1700 units/d) and increased doses of Leptin and Metformin (Table 1).

Discussion: In this case, an injection of DMPA was associated with profound changes in glucose metabolism. The metabolism of DMPA in patients with lipodystrophy is unclear. High levels of progestin immediately after injection may worsen the insulin resistance and leptin metabolism in patients with lipodystrophy, who are much more sensitive to such changes. DMPA and probably other progestin-only contraceptives should be avoided in this specific group of patients.

Laboratory Parameters and Pharmacologic Management

	Baseline (Jan 2006)	Post-DMPA (8wks)	Post-DMPA (10wks)
Fasting Glucose	100 mg/dL	310 mg/dL	101 mg/dL
Serum Insulin	43.6 unit/mL	13.4 unit/mL	NM
HgbA1c	5.6%	12.9%	NM
Triglycerides	217 mg/dL	704 mg/dL	167 mg/dL
Leptin Dose	0.7 mL BID (3.5 mg)	1 mL BID (5 mg)	1.4 mL BID (7 mg)
Metformin Dose	1000 mg	2000 mg	2000 mg
Insulin Dose	None	1700 units	350 units

340

Women's Preferred Mode of Delivery for Breech Presentation at Term. Marjolein Kok,[1] Brent Opmeer,[2] Joris van der Post,[1] Ben Willem Mol.[3] (SPON: Eric AP Steegers). *[1]Obstetrics & Gynecology, Academic Medical Centre, Amsterdam, Netherlands; [2]Clinical Epidemiology & Biostatistics, Academic Medical Centre, Amsterdam, Netherlands; [3]Obstetrics & Gynecology, Maxima Medical Centre, Veldhoven, Netherlands.*

Objective: To assess women's preferences for mode of delivery in case of breech presentation at term, and their judgement of the neonatal short- and long-term risks as well as the maternal risks.

Design*:* Trade-off interviews*.*

Setting: Three hospitals and a midwifes clinic in The Netherlands.

Population: 40 women with a fetus in breech presentation (breech group) and 40 women with a fetus in cephalic presentation (cephalic group) with a gestational age from 36 weeks onwards.

Methods*:* We offered pregnant women scenarios of vaginal and caesarean breech delivery in which one-month and two-year neonatal and maternal complication rates were varied. The baseline differences for poor outcome were set at 3% lower short-term neonatal complication rate after a cesarean delivery, no difference in neonatal complication rate after two years and a 1% higher maternal complication rate after a cesarean delivery.

The impact of the complication rates on women's preferences was visualised graphically in trade-off curves. Differences in trade-off curves were tested using the Wilcoxon signed ranks test.

Main outcome measures: Preference for vaginal or caesarean delivery; trade-off between maternal and neonatal complication rates.

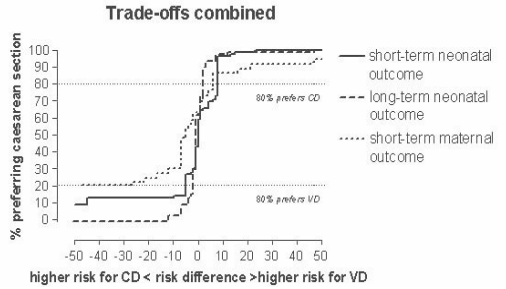
Results: Caesarean delivery was the preferred mode of delivery for 66% of the women. The one-month neonatal complication rate had to be varied between -5% and +8% until a substantial number of women changed their preference, whereas this window was only -2% to +2% for the two-year neonatal complication rate (P= .01). The impact of the maternal complication rate on the preferred mode of delivery was limited.

Conclusion: The preferred mode of delivery in women with a fetus in breech position at term is mainly dependent on the neonatal condition at two years of age.

Figure 1.

Women's preferences for a caesarean delivery (CD) relative to a vaginal delivery (VD): trade-of curves for neonatal and maternal outcomes combined in one graph.

The dotted horizontal lines represent the point where 80% prefers a caesarean and vaginal delivery respectively.



341

Plotting Contractions Using Uterine Electromyography – An Alternative to Traditional Tocodynamometry. Robert E Garfield,[*] Shao Q Shi, Lynette B MacKay, William L Maner. *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: To determine if uterine contraction events, plotted using uterine electromyography (EMG) data, correlate with tocodynamometer (TOCO)-plotted contraction events. **Materials and Methods:** 323 contraction vs. no-contraction events were observed from ten term-pregnant women. Uterine EMG was measured non-invasively from the abdominal surface of each patient for 30 minutes. TOCO was used simultaneously to measure uterine contractions. The spectral-temporal-mapping (STM) and root-mean-square (RMS) methods were applied to the uterine EMG data to generate contraction curves similar to TOCO "bell-shaped" curves. For each patient, begin and end-points of uterine electrical "bursts" and uterine contractions were determined. Correspondence between raw EMG bursts and contractions plotted by various methods was established by looking for temporal overlap. Bursts/contractions were assigned "1" and non-events (no activity in the period examined) "0." Kappa inter-rater statistic was calculated to find a measure of agreement between raw EMG and contraction-plotting methods. Correlation analysis was performed between raw EMG, TOCO, RMS and STM plots using the Pearson-product-moment test. The average percentage of burst and/or contraction-events plotted by EMG, RMS and STM compared to TOCO was found and compared using ANOVA. For all statistical tests, $P<0.05$ was considered significant. **Results:** Kappa inter-rater agreement was 0.823 between EMG, TOCO, RMS and STM. Significant correlation was found between all plots (Table: R-value/P-value shown). There was no significant difference in the relative percentage of burst/contraction events plotted by EMG, RMS, and STM compared to TOCO (EMG:114.32±18.86%; TOCO:100.00±0.00%; RMS:109.18±17.05%; STM:102.73±8.31%) **Conclusions:** Uterine EMG bursts correspond strongly to TOCO contraction plots. EMG-generated contraction plots (using RMS or STM) are statistically indistinguishable from TOCO contraction plots, although STM-plots seem to correlate better to TOCO than RMS-plots. For term patients, uterine EMG could be used in place of TOCO in the clinic for plotting contractions. Supported by NIH R01-HD037480.

TABLE

	TOCO	RMS	STM
RAW EMG	0.835 / <0.001	0.838 / <0.001	0.840 / <0.001
TOCO	- -	0.805 / <0.001	0.814 / <0.001
RMS	- -	- -	0.824 / <0.001

FRIDAY

342

Immune Cell Trafficking and Remodeling of the Extracellular Matrix in Human Cervical Tissues during Cervical Ripening and Parturition. April T Bleich,[1] Steven M Yellon,[2] Ruth A Word.*[1] *[1]Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA; [2]Physiology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

OBJECTIVE: Numerous studies have demonstrated an influx of leukocytes into the cervix during active labor. The role of immune cells in cervical ripening prior to labor, however, is not clear. The objective of this study was to determine if immune cell trafficking occurs in the human cervix during cervical ripening prior to labor. **METHODS:** Full thickness cervical sections were obtained near the internal cervical os from 28 puerperal hysterectomies conducted from 1999 - 2006 (early pregnant, n = 4; third trimester before ripening, n = 9; term with cervical ripening, n = 7; term in labor, n = 8). Status of cervical ripening was determined by modified Bishop scoring. Sections were stained for collagen using Trichrome stain. Inflammatory cells were identified by immunohistochemistry with antibodies to CD68 for macrophages and CD45 for leukocytes. One examiner blinded to clinical status counted the number of stained cells in ten randomly selected high power fields (hpf) of the subepithelium and deep stroma. **RESULTS**: Remodeling of the extracellular matrix during cervical ripening was associated with disorganization and loosening of collagen. Leukocytes in cervical stroma were similar in tissues obtained in early gestation (164 ± 71 /10hpf) or at term before cervical ripening (139 ± 26 /10hpf). Interestingly, although the number of intravascular and subepithelial leukocytes increased dramatically in cervical tissues in labor, the number of leukoctyes during cervical ripening was not significantly different from early gestation or late pregnancy (128 ± 45 /10hpf). In contrast, during cervical ripening at term prior to labor, macrophages were increased 4-fold in cervical stroma (from 14 ± 9 to 61 ± 11 /10hpf, $P \leq 0.01$) and 7-fold in the subepithelium (from 40 ± 21 to 267 ± 65 /10hpf, $P = 0.003$) and these levels were maintained in the dilated laboring cervix. **CONCLUSIONS:** Results from this study indicate that ECM remodeling of the cervix prior to uterine contractions of labor is associated with increased numbers of macrophages, but not leukocytes, in cervical stroma and subepithelium. In contrast, cervical dilation/labor is associated with leukocyte recruitment and extravasation. We suggest that remodeling of the cervix prior to and during labor involves unique processes of immune cell trafficking and activation.

343

Early Pregnancy Cholesterol and Triglyceride Concentrations Are Higher in Women with Spontaneous Preterm Birth. Janet M Catov,[1,3] Lisa M Bodnar,[1,2,3] Kevin E Kip,[1] Carl Hubel,*[2,3] Roberta B Ness,[1,2,3] James Roberts.*[1,2,3] *[1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [3]Magee-Womens Research Institute, Pittsburgh, PA, USA.*

Objective: Women who deliver preterm infants may be at increased risk later in life for cardiovascular disease, but mechanisms are not understood. The authors proposed that, similar to preeclampsia which is also associated with cardiovascular disease, women with preterm birth may demonstrate evidence of dyslipidemia.

Methods: In a nested case control study of women with spontaneous preterm birth, cholesterol, HDL, LDL, and triglycerides were evaluated in non-fasting serum. Lipid concentrations before 15 weeks, lipid changes across gestation, and risk for preterm birth were evaluated in women who delivered <34 weeks (n=23), 34-<37 weeks (n=67) and ≥ 37 weeks (n=199).

Results: Overweight women (pregravid BMI ≥25 kg/m²) who delivered <34 weeks had higher early pregnancy concentrations of cholesterol (226.1 [SD 54.5] vs. 191.4 [SD 35.7] mg/dl) and LDL (139.9 [SD 52.3] vs. 109.2 [SD 30.6] mg/dl) compared to overweight women with term births. Among overweight women, early pregnancy concentrations of total cholesterol and LDL increased as gestational age at delivery decreased (p for trend, <0.01). Lean women (pregravid BMI <25 kg/m²) with preterm birth 34 to <37 weeks had elevated triglycerides before 15 weeks compared to lean women with term births (100.4 [SD 47.9] vs. 81.7 [SD 36.0], p<0.01). There was a reduced triglyceride response in the first half of pregnancy among women who delivered <34 weeks. High cholesterol or triglycerides before 15 weeks were associated with a 2.8-fold (95% CI 1.0-7.9) and 2.0-fold (95% CI 1.0-3.9) increased risk for spontaneous preterm birth <34 weeks and 34-<37 weeks, respectively, after adjustment for race, BMI, education, and family history of hypertensive disorders of pregnancy.

Conclusion: Our results indicate the presence of dyslipidemia in women with spontaneous preterm birth. Future studies should be directed at understanding the complex underlying processes that explain these findings.

344

Matrix Metalloproteinase-8 (MMP-8) and Interleukin-6 (IL-6) Levels in Cervicovaginal Fluid Samples Are Not Associated with an Increased Risk of Spontaneous Preterm Delivery. Samuel Parry,* Rita S Leite, Yujie Ma, Dominic Marchiano. *Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: Given the relatively poor sensitivity of fetal fibronectin screening, we sought to determine if screening cervicovaginal fluid samples for inflammatory proteins that induce fetal membrane degradation could identify women at risk for spontaneous preterm delivery.

Methods: Cervicovaginal fluid was collected from women who presented to our obstetrical triage unit with uterine contractions between 20 and 32 weeks gestation. Women were excluded if their cervix was greater than 2 cm dilated, vaginal bleeding was present, or the fetal membranes were ruptured. Obstetrical outcomes were determined by chart review. MMP-8 and IL-6 levels were determined in each sample by ELISA, and MMP-8 and IL-6 levels were normalized to total protein levels (Pierce protein assay) in each sample. We estimated that we needed to enroll at least 150 women into our cohort to identify a 2.5 fold increased risk of preterm delivery in women with elevated MMP-8 or IL-6 levels (power = 0.80, alpha = 0.05).

Results: 156 women were enrolled, and 31 delivered before 37 weeks gestation (15 before 34 weeks). MMP-8 and IL-6 levels (normalized to total protein levels) were similar between women who delivered at term and women who delivered preterm. Using threshold values based on ROC curve analyses, elevated MMP-8 levels (greater than 0.5 ng/mg total protein) or IL-6 levels (greater than 1.5 pg/mg total protein) did not identify women at increased risk of spontaneous preterm delivery at less than 37 or 34 weeks (all P values were greater than 0.4).

Conclusions: MMP-8 and IL-6 levels in cervicovaginal fluid samples are not associated with the risk of spontaneous preterm delivery in women with preterm contractions. The utility of rapid MMP-8 assays to predict preterm delivery may be dependent upon increased levels of total protein in cervicovaginal fluid, but our results indicate that MMP-8 is not expressed disproportionately in cervicovaginal fluid compared to other proteins preceding spontaneous preterm delivery. Because preterm labor and delivery is multifactorial and the inflammatory response is not the only pathway leading to preterm delivery, our hypothesis that MMP-8 and IL-6 screening might be superior to fetal fibronection for predicting spontaneous preterm delivery was proven false.

345

Strong Ion Difference – A Novel Tool for Fetal Metabolic Assessment. Yoni Cohen,[1,3] Adi Nimord,[2,3] Ariel Many,[1,3] Michael Kupferminc,[1,3] Joseph Lessing,*[1,3] Jessica Ascher- Landsberg.[1,3] *[1]Lis Maternity Hospital, Tel Aviv Souraski Medical Center, Tel Aviv, Israel; [2]Intensive Care Unit, Tel Aviv Souraski Medical Center, Tel Aviv, Israel; [3]Tel Aviv University, Sackler School of Medicine, Tel Aviv, Israel.*

Objective:

Strong ion difference (SID), the difference between the sums of cations and anions in the plasma, has become a fundamental tool for acid-base status analysis in critically ill patients. SID allows a more comprehensive approach to diagnosing acid base abnormalities than the traditional approach, based only on bicarbonate and PCO_2. We sought to investigate the feasibility of the strong ion difference calculation as a novel approach to fetal acid base status evaluation, and set reference values for clinical setup.

Methods:

62 samples were drawn from the umbilical vein immediately during normal spontaneous delivery (n=32) and elective cesarean sections (CS) (n=30).

The actual SID (SIDa) and the effective SID (SIDe) were calculated according to Stewart's Principle: $([Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}]-[Cl^-]-[Lactate^-] = SIDa)$. and Fencl's Principle: $([HCO_3^-] +[Albumin^-]+[Phosphate^-])=SIDe)$.

We compared the differences in concentrations of each ion and calculated the differences in SIDa, SIDe and traditional acid base balance parameters, between spontaneous deliveries and elective CS.

Results:

1. The SIDa during normal delivery and elective CS was found to be in the range (mean±SD) of 34.5±4.02 and 36.15±2.72 mmol/L, respectively. The variations between normal delivery and CS were nearly significant (P=0.066). The SIDe between the groups was almost identical (39 mmol/L)

2. The mean base excess was higher after CS compared to normal delivery (1.19 vs 0.066 p=0.04). However pH values were similar (7.38±0.05 vs 7.37±0.05).

acid base variables

	Normal Delivery		Elective CS		p value
	Mean	SD	Mean	SD	
Lactate (mmol/L)	4.15	1.53	2.82	1.64	0.02
K (mmoml/L)	5.41	0.64	4.79	0.5	<0.001
Phosphate (mg/dL)	5.46	0.53	5	0.58	0.002
Albumin (gr/L)	37.25	2.82	34.53	2.33	<0.001
pCO2	41.48	6.29	44.55	5.93	0.055

Conclusion:

To the best of our knowledge, this is the first report on SID in umbilical vein in the obstetric literature.

Although the concentrations of Lactate, Albumin, Phosphate and K are significantly higher, and the base excess is lower, in umbilical vein blood after normal delivery compared to CS, it does not result in metabolic acidosis, since neither the differences in the SID nor in the pH were significant.

346

Safety and Efficacy of the Combination of Nitric Oxide Donor and Misoprostol for Cervical Ripening at Term. Boonsri Chanrachakul, Piyaporn Punyavachira, Domerudee Preechapornprasert, Matchuporn Sukprasert, Danunoot Tangkunmongkol, Yongyoth Herabutya. (SPON: Fiona Broughton-Pipkin). *Obstetrics&Gynecology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*

Objective: To compare the efficacy and safety of the combination of isosorbide mononitrate (IMN) and misoprostol with misoprostol alone for cervical ripening at term.

Methods: This study was approved by the Ethics Committee and informed consent was obtained from each participant. Three hundreds and ten term pregnant women with Bishop score of 6 or less were randomly allocated to recieved either 40 mg of IMN and 50 mcg of misoprostol (155) or 50 mcg of misoprostol (155) every 6 hours for the maximum of 4 doses. They were sent to labor ward for amniotomy or oxytocin if their Bishop score were 8 or more or their cervices were not ripe 24 hours after treatment. Outcomes of labor and adverse effects were assessed.

Results: The combination of IMN and misoprostol was associated with fewer episodes of uterine tachysystole (5.2% vs 0.7%; P<0.05) but the incidence of headache was more frequent than those in misoprostol group (43.7% vs 5.8%; P<0.01). Although The duration from drugs administration to the beginning of uterine contraction was shorter in misoprostol group (1.5 (1, 2.42) vs 2.08 (1.5, 4) hrs; P<0.01), the interval from starting medication to delivery between both groups was not different (11.78 (8.15, 17.36) vs 13.13 (8.72, 17.97) hrs; P=0.16).

Conclusion: The combination of IMN and misoprostol resulted in the reduction of uterine tachysystole without altering the effectiveness of misoprostol on cervical ripening.

347

A Randomized, Multicentre, Open Label Trial Comparing the Start of the Induction of Labor with Intravenous Oxytocin According to the Circadian Rhythm with Standard Care. Jannet Bakker,[1] Ben Willem Mol,[2] Rien de Vos,[3] Joris van der Post.[1] (SPON: Eric AP Steegers). *[1]Obstetrics & Gynecology, Academic Medical Centre, Amsterdam, Netherlands; [2]Obstetrics & Gynecology, Maxima Medical Centre, Veldhoven, Netherlands; [3]Clinical Epidemiology & Biostatistics, Academic Medical Centre, Amsterdam, Netherlands; [4]Obstetrics & Gynecology, Academic Medical Centre, Amsterdam, Netherlands.*

Objective To compare duration of labor and obstetrical outcomes of induction of labor with intravenous oxytocin starting in the evening (21:00 hrs) in concordance with the circadian rhythm and standard care that starts induction of labor in the morning.(07:00 hrs)

Design Prospective open label multicenter randomized clinical trial

Setting Three hospitals in Amsterdam

Patients Patients who were admitted for induction of labor with intravenous oxytocin from 1st November 2003 until 15st September 2006

Methods The primary outcome is duration of labor, for the intention to treat analysis defined as time from start of the drip until time of birth and in for the par protocol analysis as occupation of the delivery room. Secondary outcomes are number of interventions, neonatal condition, necessity for pain relief, number of intrapartum infections and patient satisfaction with quality of care.

Results We randomized 132 multiparous women and 242 primiparous women. There is no significant difference in the primary and secondary outcomes. The number of epidurals are the same in both groups but the evening group shows a significant greater need for morphine. (PP analysis in the group primiparae; RR: 0.71(CI: 0.55-0.94), p=0.019)

Conclusion

The outcome of our study shows that start of induction of labor with intravenous oxytocin round the clock is a safe procedure in terms of neonatal and maternal outcomes. To time birth during office hours one should start induction of labor of multiparous women in the morning. More than 50% of the primiparae delivers after 7:43 a.m. when induction starts in the evening.

Results intention to treat analysis

	primi N=242			**multi N=129**		
	morning group n=124	**evening group n=118**		**morning group n=63**	**evening group n=66**	
duration of labor (hh:mm, SD)	12:07(05:41)	11:26(5:19)	p=0.34	7:48(4:41)	08:41 (4:31)	p=0.77
instrumental delivery	33.1%	33.9%	p=0.89 RR=0.52	12.7%	4.5%	p=0.77 RR=2.79
necessity for painrelief	28.2%	39.8%	p=0.057 RR=0.77	4.8%	12.1%	p=0.12 RR=0.45
Apgarscore<7	1.6%	0.8%	p=0.59 RR=0.52	4.8%	1.5%	p=0.45 RR=0.45

348

The Diagnosis of Intra-Amniotic Infection (IAI) in the Setting of a "Bloody Tap". Sonya Abdel-Razeq, Irina Buhimschi, Victor Rosenberg, Michael Cackovik, Christian Pettker, Lissa Magloire, Anna Sfakianaki, Catalin Buhimschi. (SPON: Charles J Lockwood). *Ob./Gyn.&Reprod. Sci, Yale University, New Haven, CT, USA.*

Objective: Interpretation of WBC count as rapid indicator of IAI in the setting of amniotic fluid (AF) contamination with red blood cells (RBCs) –"bloody tap"– is often difficult. We examined whether contamination of AF with blood impacts on diagnostic performances of WBC count to diagnose IAI. **Methods:** 194 consecutive women pregnant with singletons presenting with preterm labor/ PPROM who had an amniocentesis to rule out IAI were enrolled prospectively. Rapid tests included WBC count, glucose, lactate dehydrogenase and Gram stain. AF was also cultured for aerobic, anaerobic, *Ureaplasma* and *Mycoplasma* species. A "bloody tap" was defined as RBC count >1000 cells/mm^3 and IAI as a positive AF culture. Analysis included ANOVA, Spearman correlation, kappa analysis of agreement. Sensitivity, specificity, positive (PPV), negative (NPV) predictive values and accuracy were calculated for each proposed WBC cut-off (30, 50, 100 cells/mm^3). **Results: 1)** The prevalence of a "bloody tap" was 25% (n=48); **2)** In a "non-bloody" tap, WBC counts were increased by IAI (WBC median[range]: IAI: 486[1-9,630] *vs.* no-IAI: 3[0-3,370] cells/mm^3, p<0.001); **3)** In a "bloody" tap the difference in WBC count with IAI did not reach significance (IAI: 133[10-132,000] vs. no-IAI: 42[2-37,000] cells/mm^3, p=0.08); **4)** There was a significantly weaker correlation of WBC count and IAI in "bloody" (r=0.257, p=0.08) *vs.* "non-bloody" samples (r=0.551, p<0.001, z statistic 2.1, p=0.03); **5)** All WBC cut-offs had significantly lower accuracies in diagnosing IAI in "bloody" compared to a "non-bloody" samples (Table), which did not improve after correction for either maternal and/or neonatal hematological indices; **6)** Of all rapid laboratory tests, Gram stain had the highest accuracy to predict IAI (82%) in a "bloody" sample. **Conclusion:** In the context of an AF sample contaminated with >1000 RBCs/mm^3, an elevated WBC count is not an accurate indicator of a positive culture result, and thus should be interpreted with caution in the absence of a positive Gram stain.

Cut-off	Sensitivity [95% CI]	Specificity [95 CI]	PPV %	NPV %	Accuracy %	*agreement Kappa*
"Bloody tap" [RBC >1000 cells/mm³]						
30 cells/mm³	79 [49-95]	44% [27-62]	37	83	54	0.170; poor
50 cells/mm³	64 [35-87]	56 [37-72]	38	79	58	0.167; poor
100 cells/mm³	50 [23-76]	68 [49-82]	39	77	63	0.163; poor
"Non-bloody tap" [RBC ≤1000 cells/mm³]						
30 cells/mm³	76 [57-88]	91 [84-95]	71	93	88	0.655; good
50 cells/mm³	64 [45-79]	94 [87-97]	75	90	87	0.632; good
100 cells/mm³	64 [45-79]	94 [87-97]	75	90	87	0.607; good

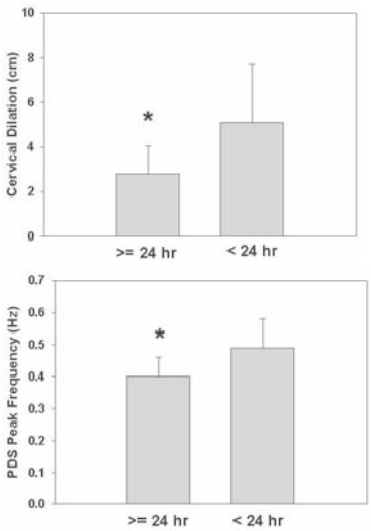
FRIDAY

349

Comparing Uterine Electromyography Measurements to Other Parturition Factors in Term Pregnant Patients. Sangeeta Jain, William L Maner, Lynette B MacKay, Shao Q Shi, Erica J Bette, Jaime D Hart, Robert E Garfield.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: To determine if uterine electromyography (EMG), specifically the power density spectrum peak frequency (PDSPF), correlates with parturition factors such as gestational age (GA), measurement-to-delivery time (MTDT), cervical dilation (CD), cervical effacement (CE), and station (S). **Materials and Methods:** Forty-five term-pregnant women were included. Uterine EMG was measured non-invasively from the abdominal surface of each patient for 30 minutes. CD, CE, and S were assessed at or near the time of uterine EMG measurement. Patients were grouped (G1:MTDT<24 hours, N=30; G2: MTDT≥24 hours, N=15). Correlation analysis was performed on all variables, using the Pearson-product-moment; t-test was used to compare groups (P<0.05 was considered significant). **Results:** Significant correlation was seen (Table: R-value/P-value shown). Significant differences were also observed between G1 and G2 for all variables except for GA (Figures). **Conclusions:** Uterine EMG activity is greater near delivery, and correlates with MTDT, CD, CE, and S, making it a viable alternative diagnostic parameter for assessing the state of parturition. Supported by grant NIH R01- HD037480.

	MTDT	GA	PDSPF	CE	S
CD	-0.450 / <0.005	0.236 / 0.149	0.684 / <0.001	0.621 / <0.001	0.717 / <0.001
MTDT	- -	-0.488 / <0.001	-0.419 / <0.005	-0.320 / 0.065	-0.423 / <0.050
GA	- -	- -	0.239 / 0.114	0.008 / 0.964	0.020 / 0.914
PDSPF	- -	- -	- -	0.484 / <0.005	0.493 / <0.005
CE	- -	- -	- -	- -	0.657 / <0.001



350

Short Inter-Pregnancy Interval Is a Significant Risk Factor for Subsequent Poor Obstetric Outcome in Patients with Prior Full Term Birth. Andrea Goldberg, Sindhu Srinivas, Michal A Elovitz.* *OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: While some studies have suggested that short inter-pregnancy interval may increase the risk for subsequent poor pregnancy outcome, few have addressed whether this possible association is confounded by maternal obstetric history. We sought to determine whether short interval to next pregnancy was independently associated with poor outcome in subsequent pregnancy, defined as second trimester pregnancy loss (STL, 14-23.6 wks), or spontaneous preterm birth (PTB, 24-36.6 wks).

METHODS: A retrospective cohort study was conducted. 3 groups of patients were identified from 2002-2005 (index pregnancy): patients with a STL (N=30), patients with a PTB (N=76), and patients with a full term delivery (FTD) (N=76). Computerized medical records and obstetric databases were used to obtain demographic, medical, and obstetrical histories, and information about subsequent pregnancy outcome. Interpregnancy interval was defined as interval from delivery date of index pregnancy to LMP of subsequent pregnancy. Significant associations were determined using Chi square. Associations of interest were then adjusted for potential confounders, including race, age, pregnancy history, and prenatal care, using multivariable logistic regression. Secondarily, a stratified analysis was performed by prior pregnancy history.

RESULTS: Independent of obstetric history, patients with an inter-pregnancy interval < 6 months were 10.1 times more likely to have poor obstetric outcome in a subsequent pregnancy (CI [1.94-52.09], p= 0.006). In patients with a prior PTB, interpregnancy interval < 6 months had no significant effect on risk of subsequent poor pregnancy outcome. In contrast women with a prior FTD, pregnancy interval < 6 months resulted in 8.9 times greater odds of having a STL or PTB in a subsequent pregnancy (CI [1.71-46.12], p= 0.009). In patients with prior STL, pregnancy interval < 6 months resulted in 4.1 times greater likelihood of poor outcome in a subsequent pregnancy (CI [0.26-64.44], p= 0.31).

CONCLUSION: Short inter-pregnancy interval significantly increases the likelihood of PTB and STL, independent of obstetric history. While the mechanisms by which short intervals result in adverse outcomes remain unclear, counseling, especially in patients with prior full term deliveries, should reflect the increased risk with inter-pregnancy interval < 6 months.

351

A Randomised Controlled Trial of Food Intake in Labour. Bing Liu,[1] Geraldine O'Sullivan,[2] Darren Hart,[1] Mark Waterstone,[3] Andrew H Shennan.[1] (SPON: Lucilla Poston). *[1]Division of Reproduction and Endocrinology, King's College London, London, United Kingdom; [2]Department of Anaesthesia, St Thomas' Hospital, London, United Kingdom; [3]Department of Obstetrics & Gynaecology, Queen Mary's Hospital, Sidcup, United Kingdom.*

OBJECTIVE: To evaluate the influence of food intake during labour on obstetric and neonatal outcome. METHODS: Between May 2001 and April 2006, we conducted a randomised controlled trial at St Thomas' Hospital and Queen Mary's Hospital London. 2405 primiparous low-risk labouring women were randomised into a 'Feeding Group' or a 'Water Only Group'. Women in the 'Feeding Group' were advised to have low-fat, low residual diet through out labour at will, while women in the 'Water Only Group' were advised to drink water only. Pre-labour food intake within 6 hours prior to the establishment of active labour was collected. Labour food intake was categorised as Nil Intake, Water, Calorific Drinks and Solids. A subgroup of participants (152) was asked to answer a post delivery questionnaire using a 10-cm visual analogue related to labour experience to evaluate the influence of food intake during childbirth on the level of maternal satisfaction towards labour. The primary outcome was the spontaneous vaginal delivery rate (SVD). Duration of labour, instrumental delivery rate, caesarean section rate, augmentation of labour, incidence of vomiting, Apgar scores and need for admission to SCBU/NICU were also evaluated. The study had 90% power to detect a 5% change in spontaneous vaginal delivery rate. RESULTS: There was no significant difference in SVD (44% in the Feeding Group vs. 45% in the Water Group; RR, 0.986; 95% CI 0.9-1.1), duration of labour (704mins vs.726mins *p=0.18)*. No difference was observed with respect to incidence of vomiting ('once', 18% vs.16%; RR, 1.09; 95% CI 0.91-1.3; *p=0.36*; 'more than once', 17% vs.17%; RR, 1.007; 95% CI 0.85-1.2; *p=0.96*), medical interventions and neonatal outcome between the two groups. However, women in the eating group rated their experience better because of eating(*p<0.001)*.CONCLUSION: Light dietary intake during labour did not influence labour and neonatal outcome. However, women who were fed felt their labour experience was better. Women should be allowed to eat in labour.

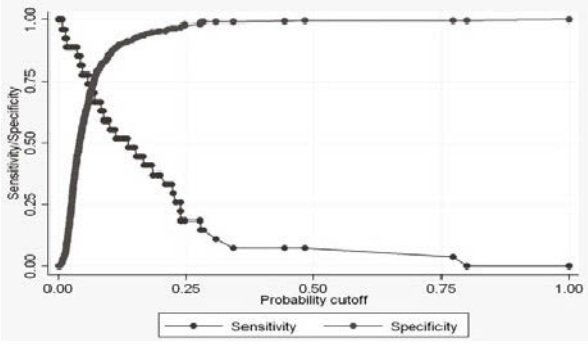
352

Rate of Change of Corticotrophin Releasing Hormone and hCG Nadir Provide Accurate Identification of Women at Risk of Preterm Birth. Roger Smith,* Julia Smith, Maria Bowman, Shaun McGrath, Warwick Giles.* *Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia.*

Recently progesterone therapy has been shown to markedly reduce preterm birth in women at high risk due to a previous preterm birth. Unfortunately there are no accurate predictors for women in their first pregnancy. We have previously shown that maternal plasma Corticotrophin Releasing Hormone (CRH) can predict preterm birth but with a relatively low sensitivity and specificity. We hypothesised that the endocrine pathways leading to uterine activation may respond to rate of change of endocrine signals rather than absolute levels and that rate of change of plasma CRH and other variables may predict preterm birth more effectively than absolute levels.

Methods: Four hundred unselected women were prospectively followed to delivery with serial maternal blood samples taken at 4 weekly intervals. Samples were assayed for plasma CRH, human chorionic gonadotrophin (hCG) and other variables. Curves were fitted to time course data for each individual and each variable. Data at 26 weeks were then generated from the fitted functions. An exponential and a quadratic curve were used to model log transformed CRH and

hCG respectively for 27 preterm and 364 singleton term pregnancies. Multiple logistic regression was performed to test the relationship between changes in the variables and the outcome of interest: preterm birth.
Results •At 26 weeks multivariate analysis revealed that a combination of Rate log CRH, log CRH and timing of HCG minimum with a probability cutoff of 0.18 produced a sensitivity of 40.74% and a specificity of 95.08% and correctly classified 91.35% of patients.
Conclusions. Like other endocrine systems it seems that gestational tissues respond to dynamic changes in regulatory processes rather than absolute concentrations. This can be exploited to more accurately predict events such as preterm birth. More accurate identification of obstetric risk may allow early intervention with preventative therapies such as progesterone supplementation.



353

Differential Overexpression of MMP-9 and Mechanical Inhomogeneity of the Chorioamnion during Premature Rupture of the Membranes. Markus M Valter,[1] Gerhard Artmann,[2] Yves Garnier,[*1] Markus Gantert,[1] Torsten Schmidt,[1] Peter Mallmann.[1] *[1]Dept. of Obstetrics and Gynecology, Univ. Hospital of Cologne, Cologne, Germany; [2]Bioengineering, FH Aachen, Aachen, Germany.*

Objective: The pathophysiology of premature rupture of the membranes (PROM) is despite recent progress still poorly understood. While infections seem to contribute to the problem, additional factors must be involved. In the present study, we have analyzed the expression of positive and negative modulators for matrix degradation and correlated the findings with histomechanical properties of the membranes as examined by a newly developed machinery called electronic cell drum.

Methods: Matrix protease or protease inhibitor molecules have been analyzed on mRNA (heteroduplex RT-PCR) and protein levels (Western blotting). Amnion and chorion have been investigated separately also with regard to their mechanical properties in an electronic laser chamber, generating, e.g., pressure-tension curves. A pressure-resistance mapping of the full chorioamnion has been performed.

Results: There was a statistically highly significant association exclusively of MMP-9 overexpression with PROM. Other protease or protease inhibitor molecules failed to demonstrate such correlation. The mechanical examinations of the membranes showed in response local differences of the physical resistance, the uterine pole being the locus of predilection for rupture. Fetal membranes from early weeks of gestation revealed higher absolute values for mechanical resistance, gradually decreasing towards term. The amnion represents a buffer system after rupture of the weaker chorion. Microfissures in the membranes can be generated through mimicking premature labor *in vitro*.

Conclusion: Our data support a key role for MMP-9 during PROM and show mechanical differences of the chorioamnion resistance according to embryonic origin of the membrane component, the gestational week and the local part of the membrane in its orientation. The newly developed machinery proves to be a powerful instrument to test membrane rupture and influencing parameters separately *in vitro*.

354

Does the Presence of Placental Infection at the Time of a Second Trimester Loss Predict Recurrent Second Trimester Pregnancy Loss? Andrea Goldberg, Sindhu K Srinivas, Samuel Parry,[*] Michal A Elovitz.[*] *OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: Although 2nd trimester loss (STL) complicates 1-2% of recognized pregnancies, it remains a poorly understood obstetrical outcome. We previously demonstrated an association between STL, histologic chorioamnionitis (HCA), and viral presence in the placenta. The objective of this study was to determine if placental infection or inflammation,and/or maternal obstetrical factors at the time of a STL modifies the risk for recurrent STL.

STUDY DESIGN: 97 patients with spontaneous STL (14-23.6 wks) were identified prospectively from 2002-2005. Computerized medical records and obstetric databases were used to identify patients with a subsequent pregnancy (N=30). Chi square analyses were performed to determine if viral or bacterial presence in the placenta, HCA, obstetric history, or clinical presentation at index STL were significantly associated with spontaneous preterm birth (SPTB, 24-36.6 wks) or recurrent STL in the subsequent pregnancy. Multivariable logistic regression was performed to adjust for potential confounders including race, maternal age, prenatal care, and obstetric history.

RESULTS: 27% of patients had recurrent STL in their subsequent pregnancy. 33% had SPTB, with 17% delivering at <34 weeks. 100% of women with a history of SPTB, prior to the index STL, had either a STL or SPTB in their subsequent pregnancy. The presence of placental viral DNA at the time of index STL significantly reduced the likelihood of STL in the subsequent pregnancy (OR 0.04 [0.003-0.526], p= 0.015). The presence of any placental bacteria at the time of index STL appears to increase the likelihood of recurrent STL (OR 6.0 [0.67-53.75], p=0.11). Neither HCA (OR 0.35 [0.06-2.10] p=0.25), nor rupture of membranes (ROM) at presentation with index STL (OR 0.62 [0.14-2.83], p= 0.54) were associated with subsequent poor outcomes.

CONCLUSION: Bacterial infection at the time of an index STL mayconfer an increased risk of recurrent STL, while viral presence in the placenta at the time of a STL may confer protection against recurrent STL, possibly secondary to acquired immunity. While the placenta is not routinely sent for viral or bacterial DNA analysis, such testing at the time of an index STL may assist with counseling about subsequent pregnancies. Research is warranted to elucidate mechanisms and offer strategies to prevent loss.

355

Differences in the Maternal Systemic Inflammatory Response May Contribute to the Ethnic Disparities in the Rate of Preterm Birth. Jimmy Espinoza,[1,2] Roberto Romero,[*1,3] Juan Pedro Kusanovic,[1] Francesca Gotsch,[1] Sonia S Hassan,[1,2] Pooja Mittal,[2] Lara Friel,[2] Offer Erez,[1] Shali Mazaki-Tovi,[2] Nandor Gabor Than,[1] Samuel Edwin,[1] Chong Jai Kim,[4] Ricardo Gomez,[5] Bo Hyun Yoon.[6] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Dept of Obstetrics & Gynecology, Wayne State Univ, Detroit, MI, USA; [3]Molecular Medicine & Genetics, Wayne State Univ, Detroit, MI, USA; [4]Dept of Pathology, Wayne State Univ, Detroit, MI, USA; [5]Dept of Obstetrics & Gynecology, Sotero del Rio Hospital, Puento Alto, Chile; [6]Dept of Obstetrics & Gynecology, Seoul National Univ, Seoul, Korea.*

Objective: Genetic factors contribute to the higher rate of preterm delivery (PTD) in African-American (AA) women than in Caucasian or Hispanic women. Studies addressing these ethnic disparities are based on case-control investigations of DNA variants or amniotic fluid (AF) concentrations of cytokines. This study was designed to determine if there are ethnic differences in the maternal systemic inflammatory response of patients with preterm labor (PTL) and intact membranes.

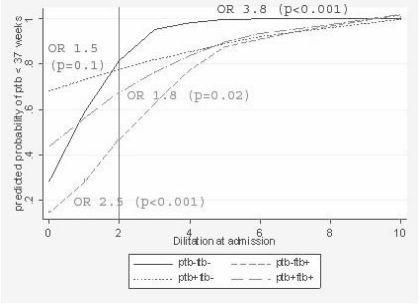
Methods: This study included AA (n=166) and Hispanic (n=93) patients with PTL in the following groups:1) Term delivery after PTL (n=109); 2) PTD without intra-amniotic infection/inflammation (IAI) (n=109); and 3) PTD with IAI (n=41). Intra-amniotic infection and inflammation were defined as positive AF cultures for microorganisms and an AF WBC ≥100 cells/ml. Maternal plasma concentrations of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, GM-CSF, and TNF-α were determined with high sensitivity multiplex assays.

FRIDAY

Results: 1) In patients with PTD without IAI, AA women had a higher median plasma concentration of IL-6 (p=0.005), IL-8 (p=0.03), IL-13 (p=0.002) and GM-CSF (p<0.001) than those of Hispanic origin; 2) In women who delivered at term, AA patients had a lower plasma concentration of IL-10 (p<0.001) than those of Hispanic origin. AA patients in this group had a higher median plasma concentration of IL-2 (p=0.03), IL-4 (p=0.02), IL-5 (p=0.04), IL-12p70 (p=0.01), and IL-13 (p=0.02). 3) In patients with PTD and IAI, there were no significant differences in the maternal plasma concentration of these cytokines between AA and Hispanic patients.
Conclusions: Differences in the maternal systemic inflammatory response to insults other than IAI may contribute to the disparities in PTL and PTD between women of AA and those of Hispanic origin.

356

Preterm Birth: Weighing the Effect of Obstetric History and Cervical Dilatation at Preterm Labor Admission. Jamie Bastek,[1] Mary Sammel,[2] Sindhu Srinivas,[1] Michal A Elovitz.*[1] *[1]OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA.*
OBJECTIVE: A history of prior preterm birth (PTB) and an advanced preterm cervical dilatation are known risk factors for PTB. How these clinical variables effect PTB risk in patients admitted with preterm labor (PTL) has not been fully explored. The objectives of this study were 1) to investigate whether prior obstetric history (OB hx) or cervical dilatation on PTL admission were significantly associated with PTB of <34 and <37 wks gestation and 2) to determine if there is a significant interaction between these variables and subsequent PTB.
STUDY DESIGN: A retrospective cohort analysis was performed of patients admitted to L&D (2002-2005) with a diagnosis of PTL at <34 wks (n=260). Obstetric history, serial cervical exams, hospital course, and gestational age at delivery was recorded. By OB hx, 4 groups of patients were identified: 1) patients with no prior PTB and no full term delivery (FTD); 2) patients with a prior FTD and a PTB; 3) patients with prior FTD and no PTB and 4) patients with no prior FTD or prior PTB. Significant associations were determined using Chi-square analyses. Multivariable logistic regression was used to control for confounders.
RESULTS: 42% of patients and 62% delivered at <34 and <37 wks respectively. For all OB hx groups, for every 1 cm increase in admission cervical exam, there was a 2.2 increase in the odds of PTB < 37 wks (P<0.0001). Controlling for race, maternal age, and prenatal care, a history of only prior PTB confered an 11-fold increase for PTB <34 wks (p=0.01). At the same cervical dilatation, the risk of PTB at < 37 wks is significantly different between all OB hx groups.
FIGURE
CONCLUSION: Regardless of OB hx or cervical exam, admission for PTL at a tertiary care center is associated with high rate of PTB. Excluding patients with only a prior PTB, admitting cervical exam can modify PTB risk for other OB hx groups. Applying these data prospectively may identify which patients are at greatest risk and lead to more appropriate use of interventions.



357

Women with Preterm Labor Have Lower Plasma Concentrations of Tissue Factor Pathway Inhibitor and Thrombin Activatable Fibrinolysis Inhibitor. Offer Erez,[1] Nandor Gabor Than,[1] Tinnakorn Chaiworapongsa,[2] Jawed Fareed,[3] Debra Hoppensteadt,[3] Jimmy Espinoza,[2] Juan Pedro Kusanovic,[1] Francesca Gotsch,[1] Bo Hyun Yoon,[4] Sonia Hassan,[2] Roberto Romero.*[1,5] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [3]Department of Obstetrics and Gynecology, Loyola University Medical Center, Maywood, IL, USA; [4]Department of Obstetrics and Gynecology, Seoul National University, Seoul, Korea; [5]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA.*
Objective: Preterm labor (PTL) has been associated with increased thrombin generation. Human decidua is a rich source of tissue factor (TF) and a disorder of decidual hemostasis has been proposed as a cause of premature labor and delivery. The aim of this study was to determine whether PTL is associated with changes in maternal plasma concentrations of TF and tissue factor pathway inhibitor (TFPI). We studied thrombin activatable fibrinolysis inhibitor (TAFI) because this protein plays a role in coagulation, regulation of inflammation and tissue repair.
Methods: A cross-sectional study included the following groups: 1) PTL (n=138); 2) normal pregnancy (NP) (n=71). The PTL group was divided into: 1) patients with PTL without intraamniotic infection or inflammation (IAI) who delivered preterm (n=54); 2) women with PTL and IAI who delivered preterm (n=35); 3) women with PTL who delivered at term (n=49). Plasma concentrations of TF, TFPI and TAFI were measured by ELISA.
Results: 1) Maternal plasma concentrations of TF were not changed in PTL even in the presence of IAI; 2) in contrast, women with PTL had a significantly lower median TFPI maternal plasma concentration than women with NP; 3) women with PTL had a significantly lower median concentration of TAFI than NP women (Table).
Conclusion: 1) The maternal plasma concentration of TF is not changed in PTL, regardless of the presence or absence of IAI; 2) In contrast, the concentrations of TFPI were decreased; 3) Similarly, the maternal plasma concentrations of TAFI were significantly decreased.

	Normal pregnancy n = 71	PTL no IAI n =54	PTL with IAI n =35	PTL delivered at term n =49
TF (pg/ml)	345.7 (22-2660)	272.97 (34-1775)	291.56 (56-1237)	258.6 (66-1495)
TFPI (ng/ml)	66.7 (37-87)	56.65 ** (30-79)	55.2 ** (32-97)	54.7** (27-96)
TAFI (µg/ml)	99.6 (57- 58)	87.755 ** (45-186)	87.7 * (34-115)	80.8 ** (41-125)

Median (range) Significant comparisons were between normal pregnancy and PTL groups
**P<0.001,*P<0.01

358

The Preterm Prediction Study: Fetal Gender and Prematurity. Edward K Chien.* *For the NICHD MFMU Network, Bethesda, MD.*
Objective: Fetal male gender has been reported as a risk factor for preterm birth in singleton and twin gestations. The biological basis is unclear although androgens may play a role. Androgens can induce cervical ripening, and the 5α-reductase and apolipoprotein E knockout mice have defects in androgen metabolism associated with failed cervical remodeling and parturition. Our objective was to determine if fetal gender was associated with spontaneous preterm birth using data from an observational cohort study of preterm birth prediction.
Methods: Between 1992 and 1994, a prospective observational study was performed to evaluate the risk factors associated with spontaneous preterm birth at 10 university centers. This secondary analysis was performed on the entire data set. The effect of gender on gestational age at birth (categorized into ≥ 37, 32 to 36-6/7, < 32 wks) was determined using univariate and multivariate analyses (logistic regression adjusted for maternal age, maternal race, parity and smoking).

Results: Of 3,000 births, 49.8% had a male fetus and 433 births were preterm.

Relationship of Gender on Preterm Birth, Spontaneous Preterm Birth (SPTB), and PROM

	Preterm		SPTB		PROM	
	<37 weeks	<32 weeks	<37 weeks	<32 weeks	<37 weeks	<32 weeks
Male	214(14.3%)	44(2.9%)	152(10.2%)	29(1.9%)	72(4.8%)	14(0.9%)
Female	219(14.5%)	34(2.3%)	156(10.4%)	20(1.3%)	59(3.9%)	6(0.4%)

SPTB includes spontaneous preterm labor and PROM.

Logistic Regression for the Effect of Male Gender on SPTB or PROM < 32 Weeks

	SPTB			PROM		
	OR	95% CI	P-value	OR	95% CI	P-value
Unadjusted	1.47	0.83-2.61	0.19	2.37	0.91-6.17	0.08
Adjusted*	1.49	0.84-2.65	0.17	2.37	0.91-6.18	0.08

*Adjusted for maternal age, maternal race, parity, and smoking.

Discussion: We found no association between male gender and risk for preterm or spontaneous preterm birth.

359

Sonographic Myometrial Thickness in Twin Gestations: Preterm Delivery Versus Term Delivery. Anna K Sfakianaki, Christian M Pettker, Lissa K Magloire, Benjamin D Hamar, Irina A Buhimschi, Catalin S Buhimschi. (SPON: Charles J Lockwood). *Obstetrics, Gynecology and the Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

OBJECTIVE: Conventional wisdom holds that the pathophysiology of preterm birth in twin gestation is excess myometrial stretch. We postulate that in twin pregancy a thin myometrium predicts women destined to deliver preterm versus those who ultimately deliver at term. The purpose of this study was to to predict preterm birth in twin gestations, by evaluating longitudinally the in vivo changes in myometrial thickness (MT), a potential marker of stretch.

STUDY DESIGN: Abdominal ultrasound was performed prospectively in 93 women pregnant with twins. The myometrium was defined sonographically as the echo homogeneous layer between the serosa and the decidua. MT was measured by standardized protocol, at the lower uterine segment (LUS), anterior, and fundal uterine walls. Measurements were obtained during each trimester (first <14 weeks, second 14-28 weeks, third ≥28 weeks). Preterm delivery was defined as < 35 weeks' gestation.

RESULTS: 39 women delivered preterm (median [range], GA: 31 1/7 weeks [20 0/7 - 34 6/7]). 54 women had uncomplicated pregnancies and delivered at term (GA: 36 5/7 weeks [35 0/7 to 39 5/7]) (Mann-Whitney Rank Sum, $p<0.001$). There was a statistically significant difference in MT in the different uterine sites with advancing gestation ($p<0.001$, 3-way ANOVA). There was significant thinning in the LUS with advancing gestation, with the third trimester LUS being the thinnest site in both term and preterm cohorts ($p<0.05$). Comparison of term and preterm cohorts did not reveal significant differences in MT thickness at the LUS ($p=0.845$).

CONCLUSION: Twin pregnancy is characterized by thinning of the LUS during gestation in both women who deliver at term and those who deliver preterm. LUS thickness cannot discriminate between the two groups.

360

Fetal Pulmonary BMP Signalling in Hypoxic Pregnancy in Rats. JA Hansell, L Long, AS Thakor, V Johnson, NW Morrell, Dino A Giussani.* *Physiology and (2) Medicine, Cambridge, United Kingdom.*

Introduction: Human and experimental studies show that compromised fetal oxygenation during pregnancy can reduce fetal lung growth and alter its development, with adverse consequences for postnatal respiratory health (Maritz et al. *Early Hum Dev* **81**:763-771, 2005; Haworth et al. *Sem Neo* **8:** 1-8 2003). The mechanism via which intrauterine hypoxia affects fetal lung growth and development remains unknown. Bone morphogenetic proteins (BMPs) exert their effects by signalling to target genes involved in arresting the cell cycle and, hence, act to reduce cell proliferation and tissue growth (Zhang et al. *Am J Physiol* **285:** L740-L754, 2003). This study tested the hypothesis that alterations in BMP signalling contribute to the mechanisms affecting fetal lung growth and development in pregnancies complicated by reduced fetal oxygenation. The hypothesis was tested by investigating, at the end of gestation, the effects on fetal pulmonary expression of key BMP signalling proteins of hypoxic pregnancy in rats.

Methods: On day 6 of pregnancy, Wistar rats were randomised into 2 groups: maternal normoxia (n=9) and maternal hypoxia (13% O_2 from day 6-20 of gestation; term 21 days, n=8). Food and water intake was monitored daily. On day 20, dams were anaesthetised, the lung of two male fetuses within any one litter were isolated, weighed and frozen for analysis. Total RNA was extracted from 8 homogenised lungs from each group and RT-PCR was performed on various components of the BMP signalling pathway.

Results: In the fetal lung, hypoxia caused a general increase in the protein expression of several key markers of BMP signalling, only reaching significance in the expression of Smad 7 (Fig. 1). Hypoxia had no effect on maternal food and water intake or on fetal lung weight.

Conclusion: Increased pulmonary BMP signalling may contribute to the mechanisms affecting fetal lung development in pregnancies complicated by reduced fetal oxygenation.

Supported by The Lister Institute for Preventive Medicine and The British Heart Foundation.

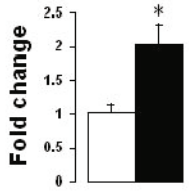


Fig.1. Effect of chronic maternal hypoxia on fetal lung BMP signalling. Data are mean+SEM of 6 fetal lungs in normoxic (□) or hypoxic (■) pregnancy. *, $P<0.05$, *t* test.

361

Fetal Origin of Heart Disease during Development at High Altitude Involves Cardiac Oxidative Stress. AD Kane,[1] CE Salinas,[2] FP Wooding,[1] CE Blanco,[2] Dino A Giussani.*[1] *[1]Physiology, Cambridge; [2]IBBA, Bolivia; [3]Pediatrics, Maastricht.*

In complicated pregnancy, fetal hypoxia as well as undernutrition is an important trigger for the developmental programming of disease. Observations in human infants at high altitude and experimental studies in rat and chick embryos show that early development under hypobaric or isobaric hypoxic condtions, results in fetal growth restriction and induces an increase in aortic wall thickness (Giussani, 2006. In: *Developmental Origins of Health and Disease*. Ed. Gluckman and Hanson, CUP. pp178-190). However, the mechanism mediating these effects remain unknown, preventing the identifcation of plausible clinical intervention. We hypothesize that oxidative stress in the fetal cardiovascular system underlies the molecular basis via which prenatal hypoxia contibutes to an early origin of heart disease. If true, treatment with antioxidants of pregnancies complicated by fetal hypoxia may prevent the early origin of heart disease. In this study we investigated whether incubation of chick embryos at high altitude is associated with an increase in cardiac oxidative stress.

Methods: Fertilized leghorn chicken eggs native to the low or highlands of Bolivia were incubated (60% humidity, 38C) at sea level (Santa Cruz, 400 m) or high altitude (La Paz, 4000 m) to yield 4 groups: sea level eggs incubated at sea level (SLSL) or high altitude (SLHA) and high altitude eggs incubated at high altitude (HAHA) or sea level (HASL). One day prior to hatching, the embryos were removed from the shell and the hearts were isolated, fixed and sectioned. Slices were stained for computerized morphometry and immunolabelled with nitrotyrosine, as a footprint for peroxinitrite generation.

Results: Embryos incubated at altitude showed an increase in the wall thickness and in the expression of nitrotyrosine in the left ventricle relative to sea level embryos (SLSL=939±30; SLHA=1268±54; HAHA=1374±49; HASL=1158±105, wall width as a % of heart weight and Figure 1; all $P<0.05$, ANOVA with Tukey test).

Conclusion: Developmental hypoxia induces an increase in cardiac oxidative stress.

The Lister Institute for Preventive Medicine.

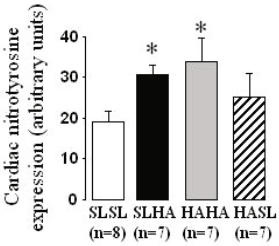


Fig.1. Effect of high altitude incubation on the mean (+SEM) expression of nitrotyrosine in the chick embryo heart. *P<0.05 *vs* SLSL.

FRIDAY

362

Fetal Origins of Cardiovascular Disease: The Role of Prenatal Hypoxia and Oxidative Stress. R Gottschalk, T Cindrova-Davies, O Spasik-Boskovic, AS Thakor, J Mullender, GJ Burton, NW Morrell, Dino A Giussani.* *Physiology and (2) Medicine, Cambridge, United Kingdom.*

The mechanisms underlying an early origin of cardiovascular disease in complicated pregnancy remain unknown, preventing the identification of targets for clinical intervention. We show in rat pregnancy that developmental hypoxia can trigger a prenatal origin of cardiovascular disease, secondary to oxidative stress. Antioxidants may thus offer plausible therapy against early origins of heart disease in pregnancy complicated by developmental hypoxia.

Methods: Group 1 (control pregnancies, 21% O2, 7 litters), Group 2 (chronic isobaric hypoxia, 13% O2 day 6-20 of gestation; term 21 d, 8 litters). Group 3 (chronic hypoxia with vitamin C in the maternal drinking water, 500 mg/100ml/day day 6-20 of gestation; 6 litters). Food and water consumption was monitored. On day 20 dams were anaesthetised, pups weighed and measured, and haematocrit noted. The fetal heart and descending aorta were fixed or frozen. No more than two pups of the same sex within any one litter were investigated. Hearts were analysed by Western blotting for HSP70, a marker of oxidative stress[1]. Aortic wall : lumen area ratio was calculated and vessels were immnunolabelled with nitrotyrosine, as a footprint of peroxynitrite generation[1].

Results: Maternal food or water intake was not affected. Hypoxic pups with (40±1%, n=6) and without (41±1%, n=8) vit C had a higher haematocrit than normoxic pups (34±1%, n=7; P<0.05). Relative to normoxic pups (n=64), hypoxic pups (n=68) had a reduced ponderal index (PI; 6.9±0.9 *vs*. 7.8±1.4; values x 100000), increased head diameter : body weight ratio (HD/BW, 2.6±0.03 *vs*. 2.4±0.03), enhanced cardiac HSP70 (Fig. 1A), and aortic thickening with greater nitrotyrosine (Fig. 1B). Treatment with vit C prevented the effects of hypoxia during pregnancy (PI = 7.8±1.1; HD/BW =2.3±0.02 (n=67) and Figure 1; all P<0.05).

Conclusions: Developmental hypoxia and oxidative stress can trigger a fetal origin of cardiovascular disease.

Supported by The Lister Institute and The British Heart Foundation

1. Halliwell B & Gutteridge JMC (1999). Free radicals in Biology and Medicine. OUP

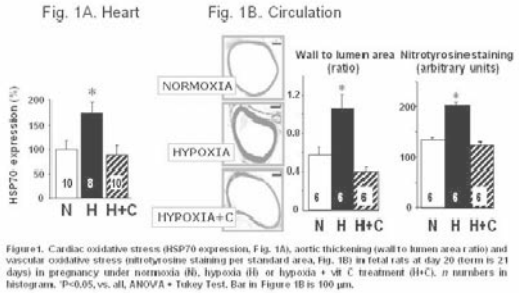


Figure1. Cardiac oxidative stress (HSP70 expression, Fig. 1A), aortic thickening (wall to lumen area ratio) and vascular oxidative stress (nitrotyrosine staining per standard area, Fig. 1B) in fetal rats at day 20 (term is 21 days) in pregnancy under normoxia (N), hypoxia (H) or hypoxia + vit C treatment (H+C). *n* numbers in histograms. *P<0.05, vs. all, ANOVA + Tukey Test. Bar in Figure 1B is 100 μm.

363

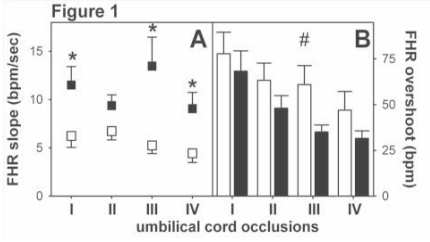
Effects of Chronic Mild Hypoxemia on Heart Rate Responses to Umbilical Cord Occlusion in near Term Fetal Sheep. Victor M Pulgar, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston Salem, NC, USA.*

OBJECTIVE: Decelerations and accelerations in fetal heart rate (FHR), observed during experimentally-induced asphyxia, are considered indicators of chemoreceptor response and myocardial stability. We have previously shown that 5 days chronic hypoxemia produces hypertension and fetal bradycardia. Following umbilical cord occlusion (UCO), hypoxic fetuses exhibited a more pronounced bradycardia and hypotension. AIM: In the present study we evaluated the characteristics of the FHR pattern in response to UCO; 1) the initial decelerations and 2) the accelerations (overshoot) after release of umbilical cord.

METHODS: At 125±1 days fetuses were submitted to 5 d of hypoxemia (75% of baseline arterial blood PO_2). After 5 d of hypoxia four 5-min UCO with 30 min recovery in between were performed in control (n=5) and hypoxic animals (n=5). The slope of the deceleration and the time to the minimum FHR during the first minute of each UCO were calculated. The FHR acceleration after occlusion was calculated as the difference between baseline and FHR maxima during the first min after releasing the occluder. Data are shown as mean±SEM and were analyzed by two way ANOVA.

RESULTS: A profound bradycardia was observed in all UCO. During the 4 different UCO, the slope of the initial bradycardia was significantly steeper in the hypoxic fetuses (hypoxic 10.8±1 bpm/sec *vs* control 5.7±0.5 bpm/sec; *P*<0.05, Figure1A) therefore the time to reach this minimum was shorter (*P*<0.05). A FHR overshoot was observed in all 4 UCO in both groups. The magnitude of the overshoot decreased with each subsequent occlusion, with the decrease being more marked in the hypoxic fetuses revealed as a significant treatment effect (# *P*<0.05, Figure 1B).

CONCLUSIONS: Chronic mild hypoxemia in near-term fetal sheep increases chemoreflex responses to a subsequent acute hypoxic insult. With repeated 5-min complete occlusions the fetal heart became less responsive to β adrenergic stimulation after insult. Funded by NIH HD37885.



364

Developmental Hypoxia and Oxidative Stress in the Early Origin of Metabolic Disease. V Kirthi, S Ekizoglou, AS Thakor, NW Morrell, SE Ozanne, Dino A Giussani.* *Physiolgy, (2) Clin Biochem and (3) Medicine, Cambridge, United Kingdom.*

Introduction: Overwhelming evidence derived from human and animal studies links development under sub-optimal conditions with insulin resistance and diabetes (Hales and Barker. *Diabetologia* **35**:595, 1992). However, the mechanisms underlying this association remain unknown, preventing clinical intervention. We tested two inter-related hypotheses that : 1) developmental hypoxia contributes to an early origin of metabolic disease; and 2) oxidative stress underlies the molecular basis via which this association occurs. The hypotheses were tested by investigating the effects of hypoxic pregnancy with and without maternal antioxidant treatment in rats on fetal hepatic expression of key insulin signalling proteins.

Methods: On day 6 of pregnancy, Wistar rats were randomised to three treatments: Normoxia (21% O2, 7 litters), Hypoxia (14% O2, 8 litters) and Hypoxia + Antioxidant (14% O2+ 500mg/100 ml/d vitamin C in the drinking water, 6 litters). Water and food intake were monitored daily. On day 20, dams were anaesthetised, the pups removed and the fetal liver isolated, weighed and frozen. Only livers from two male pups from any one litter were investigated. Frozen livers from 8 male pups per group were then randomly selected, their protein extracted and analysed by Western blotting for the expression of insulin receptor beta-subunit (IRb), p85alpha and PKCzeta.

Results: Maternal food or water intake was not affected. Fetal livers from hypoxic pregnancies showed significant upregulation of IRbeta and PKCzeta, but not p85alpha, relative to fetal livers from normoxic pregnancies. Vitamin C prevented these effects (Fig.1).

Conclusions: Increased expression of hepatic insulin receptors and PKCz in this model of adverse intrauterine conditions may provide a mechanism by which developmental hypoxia leads to increased risk of type 2 diabetes in the offspring. Antioxidant therapy in pregnancies complicated with reduced oxygen delivery to the unborn child, such as during placental insufficiency, may prevent an early origin of metabolic disease.

Supported by The Lister Institute and The British Heart Foundation.

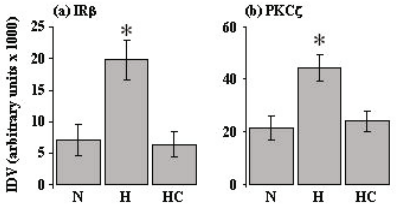


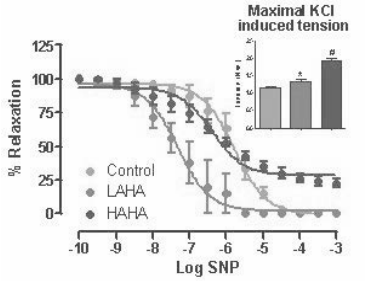
Fig. 1. Chronic maternal hypoxia results in over-expression of key proteins of the insulin-signalling pathway in fetal liver. Data are mean±SEM of 8 fetal livers in normoxic (N), hypoxic (H) or hypoxic pregnancies treated with vitamin C (HC). *P<0.05 *vs.* all, ANOVA. IDV, integrated density value.

365

Nitric Oxide (NO) Relaxing Tone in Pulmonary Arteries from Newborn Sheep Whose Gestation Took Place in Chronic Hypoxia. Bernardo Krause,[1] Emilio Herrera,[1,3] German Ebensperger,[1] Raquel Riquelme,[2] Gertrudis Cabello,[4] Victor Reyes,[1] Dino Giussani,[*5] Carlos Blanco,[7] Mark Hanson,[*6] Anibal Llanos.[1,3,4] *[1]Facultad de Medicina; [2]Facultad de Ciencias Químicas y Farmacéuticas; [3]INCAS, U. Chile, Chile; [4]CIHDE, U. Tarapacá, Chile; [5]U. Cambridge, United Kingdom; [6]U. Southampton, United Kingdom; [7]U. Maastricht, Netherlands.*

Fetal chronic hypoxia at sea level could produce pulmonary hypertension in the neonatal period. This hypertension could be the consequence of a predominance of vasoconstrictors over the vasodilators agents in the pulmonary circulation. **Hypothesis.** Pulmonary arteries from newborn sheep whose gestation took place totally or partially in chronic hypoxia have a decreased response to NO-induced relaxation and this effect is related to the stage of fetal development at which the hypoxia started. **Methods.** Utilizing wire myography we studied the contractile response to KCl and vasodilator action of SNP in pulmonary arteries from 5 newborn sheep in which total (HAHA) and 5 partial (70% of gestation, LAHA) gestation took place at 3,600m above sea level. These responses were compared with a control group from sea level (n=5). Additionally, cGMP independent NO-relaxation was determined with ODQ, a specific soluble guanylate cyclase (sGC) inhibitor. **Results.** The maximal response to KCl was increased in LAHA and HAHA newborn sheep, being the last higher than LAHA and control groups. Maximal relaxation to SNP was decreased about 30% in HAHA animals, whilst LAHA showed a similar response than controls. However LAHA and HAHA newborn showed an increased sensitivity to NO. Only LAHA newborn relaxed to SNP in presence of sGC inhibition. **Conclusions.** The NO response of the small pulmonary arteries obtained from newborns whose gestation took place in chronic hypoxia depends on the stage of fetal development at which the hypoxic insult started. Furthermore, chronic hypoxia during gestation may have an influence on the different pathways sensitive to NO, such as sGC and potassium channels.

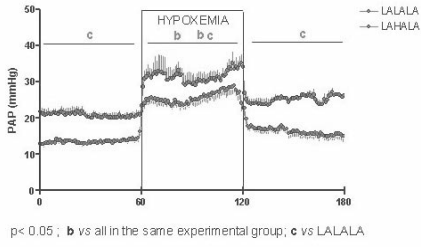
Supported by FONDECYT 1050479-Chile, The Wellcome Trust CRIG 072256/2/03/2.



366

Chronic Fetal Hypoxia during Gestation Increases the Pulmonary Artery Pressure in the Newborn Sheep. German Ebensperger,[1] Emilio A Herrera,[1,3] Raquel A Riquelme,[2] Bernardo J Krause,[1] Renato Ebensperger,[1] Roberto V Reyes,[1] Emilia M Sanhueza,[1] Gertrudis Cabello,[4] Dino A Giussani,[*5] Julian T Parer,[*6] Carlos E Blanco,[7] Mark A Hanson,[*8] Anibal J Llanos.[1,3,4] *[1]ICBM, Fac. Medicina; [2]Fac. Ciencias Químicas y Farmaceuticas; [3]INCAS, U Chile; [4]UTarapacá & CIHDE, Chile; [5]UCambridge, United Kingdom; [6]UCSF, USA; [7]UMaastricht, Netherlands; [8]U Southampton, United Kingdom.*

A crucial adaptation in the transition from fetus to newborn is the decrease in pulmonary arterial pressure and vascular resistance. Pulmonary hypertension is a frequent observation in adult mammals of lowland species at high altitude. However, little is known regarding the pulmonary circulation in newborn sheep whose gestation partly took place at high altitude. **Hypothesis:** We hypothesize that partial gestation at high altitudes causes pulmonary arterial hypertension in the newborn sheep (NB), and that this condition is also observed once the lambs are brought to sea level. **Methods:** Under ketamine anesthesia (15 mg/kg im) 14 NB, whose gestation and birth occurred in lowland (LALALA, Lluta, 50m), and 4 NB, with 70% gestation and birth in highland (LAHALA, Putre, 3,600m) were instrumented with femoral artery and vein catheters and a Swan-Ganz catheter in the pulmonary artery. All procedures and studies were performed at sea level. The studies commenced at least 3 days after surgery, between 8-12 days of age. All experiments were based on a 3h protocol divided into three periods: 1h of normoxemia, 1h of hypoxemia and 1h of recovery. We measured blood gases, pulmonary arterial pressure (PAP), cardiac output (CO), and calculated pulmonary vascular resistance (PVR). **Results:** In LAHALA neonates, PAP and CO were higher than controls, basally and during hypoxia and recovery. **Conclusions:** Partial gestation in chronic hypoxia is enough to increase the PAP. These increases may be linked to vascular remodeling in the pulmonary artery and a predominance of vasoconstrictors over the vasodilators agents in the pulmonary circulation. Studies are in progress to investigate these possibilities. *Supported by FONDECYT 1050479-Chile.*



p< 0.05 ; **b** *vs* all in the same experimental group; **c** *vs* LALALA

367

Early Neurochemical Changes in Rats Following Perinatal Asphyxia Detected by In Vivo Proton MR Spectroscopy. April E Ronca,[*1] Todd F Atwood,[2] Gale A Kleven,[3] Christina Tulbert,[4] Rebekah F Jordan,[4] Jian-Ming Zhu.[5] *[1]Obstetrics & Gynecology, Neurobiology & Anatomy, Neuroscience Program; [2]Radiation Oncology, Biomedical Engineering; [3]Obstetrics & Gynecology, Neuroscience Program; [4]Obstetrics & Gynecology; [5]Radiation Oncology, Radiation, Biomedical Engineering.*

Objective. Perinatal asphyxia is a primary determinant of neurological morbidity and mortality in human fetuses that can occur even during unremarkable pregnancies. Resulting encephalopathies often cause adverse effects on neurological development and behavior persisting into adulthood. In the present study, we exposed fetal rats to controlled levels of birth asphyxia and analyzed postnatal neurochemical changes in the striatum and hippocampus. **Methods.** Gestational day 22 pregnant rat dams were administered spinal anesthesia and the uterus externalized into a heated (37.5° C) saline bath. The blood supply feeding one uterine horn was occluded for 12min (birth asphyxia condition) while the other uterine horn remained undisturbed (control condition). Fetuses were delivered by cesarean section then cross-fostered to newly parturient dams. High resolution in vivo proton MR spectra were acquired beginning on postnatal day 7 using a 7T small animal MRI scanner. Single-voxel MRS imaging was carried out by applying the point-resolved spectroscopy (PRESS) sequence, and positioning of MRS voxels were based on T2-weighted images. **Results.** As early as Postnatal day 7, we accurately detected from the MR spectra metabolites associated with neuronal density, integrity, and function, including choline-containing compounds (Cho), N-acetyl aspartate (NAA), glutamate and glutamine (Glx), taurine (tau), and myo-inositol (mI). Each neurometabolite, derived from spectral analysis, was compared as a ratio (corrected for creatine + phosphocreatine [Cr + pCR]). We observed a significant increase in NAA in perinatally asphyxiated, as compared to non-asphyxiated control rats. **Conclusion.** In vivo MRS is a sensitive imaging tool for detecting major neurochemical changes in early life, and can be used as a non-invasive method to study neurodevelopment in relation to birth asphyxia.

Supported by WFUSM Small Animal MR Imaging Facility, WFUSM Parker Neurosciences Research & Venture Funds, and NIH Grant HD050201.

368

Amniotic Fluid S100B Protein Is Elevated in High-Risk Pregnancies with Chronic Fetal Hypoxia. Kari Teramo,[*1] Mikko Loukovaara,[1] Henrik Alfthan,[2] Esa Hamalainen,[2] Vedran Stefanovic,[1] Sture Andersson.[3] *[1]Obstetrics and Gynecology, University Central Hospital, Helsinki, Finland; [2]Clinical Laboratory Diagnostics, University Central Hospital, Helsinki, Finland; [3]Pediatrics, University Central Hospital, Helsinki, Finland.*

Objective: Increased fetal erythropoietin (EPO) levels are indicative of chronic fetal hypoxia and are associated with an increased frequency of severe neonatal complications in high-risk pregnancies. Experimental studies have shown that EPO, in addition to its erythropoietic function, has neuroprotective properties in the brain. The S100B protein is highly concentrated in the brain and has been used as a serum marker of brain damage in adults. Amniotic fluid (AF) S100B concentrations have been reported to increase in cases with fetal asphyxia or adverse neonatal outcome. However, the importance of AF S100B as a marker of neonatal brain damage has been questioned. Our aim was to study possible associations between AF S100B levels and chronic fetal hypoxia as indicated by increased AF EPO in pregnancies complicated by insulin-treated diabetes (n=22) or hypertension (n=6).

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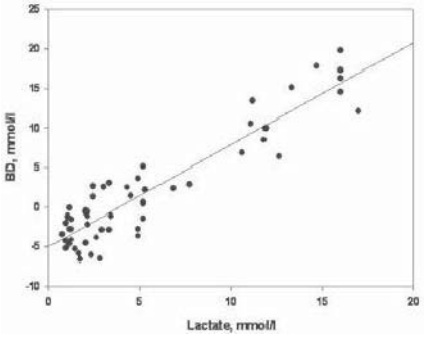
Methods: AF samples for EPO and S100B protein measurements were obtained in 28 high-risk pregnant women, in which amniocenteses were done for fetal lung maturity assessment. AF EPO and S100B protein concentrations were measured by immunoassays (Immulite EPO, Diagnostic Products, Los Angeles, CA and Elecsys S100, Roche Diagnostics, Mannheim, Germany, respectively). The median (range) of AF EPO levels in 19 healthy controls was 6.3 (1.7-13.7) mU/ml. AF EPO levels >50.0 mU/ml (>8 x median of controls) were considered to indicate chronic fetal hypoxia.
Results: AF EPO levels >50.0 mU/ml were found in 9 (32%) high-risk pregnancies. AF S100B protein concentration correlated positively with simultaneously obtained AF EPO levels (r=0.55, p=0.003) and negatively with birth weight z-score (r= -0.49, p=0.008) and placental weight (r= -0.44, p<0.02), but not with umbilical artery pH, pO_2, BE, hemoglobin or Apgar scores at birth.
Conclusions: The positive correlation of AF S100B protein with AF EPO is a new observation and suggests that high AF S100B protein levels are associated with perinatal complications. Whether increased S100B levels result from perinatal brain damage secondary to chronic hypoxia or from the placenta or other sources needs further elucidation.

369

Correlation of Base Deficit (BD) and Lactate Following Fetal Repetitive Umbilical Cord Occlusion (UCO). Michael G Ross,[*1] Martin G Frasch,[2] Robert Gagnon,[*2] Bryan S Richardson.[*2] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Ob/Gyn, Univ. of W. Ontario, London, ON, Canada.*
Objective: Fetal asphyxia-mediated metabolic acidosis results decreases pH and increases BD and lactate. The relation between fetal lactate and BD is controversial with some studies suggesting production of metabolic acids other than lactate. We sought to determine the relation between fetal BD and plasma lactate in response to UCO.
Methods: Ovine fetuses (N=6, 129±2 d) were chronically prepared with fetal brachial artery catheters and an inflatable umbilical cord occluder. Following a minimum 3 d recovery from surgery, fetuses underwent a series of mild (45s duration-5min apart), moderate (1min-3min apart) and severe (1min-2min apart) hourly series of UCO. Fetal arterial blood samples were drawn at baseline, at the end of the 1st and last UCO of a series, and at 20 and 40 min during moderate and severe UCO (1 min after UCO). Whenever the targeted pH<7.0 was detected and sustained, the UCO series were terminated.
Results: Repetitive fetal UCO as studied resulted in development of marked acidosis (pH 7.37±0.03 to 6.92±0.08; BD -3.9±1.9 to 16.3±2.7 mmol/l). Fetal plasma lactate was highly correlated with BD (R = 0.925, p<0.001 (Figure)). At time of maximal acidosis post UCO there was no change from baseline in fetal arterial pO2 (18.3±2.7 to 18.0±2.4 mmHg) although fetal pCO_2 was significantly increased (52.0±2.9 to 72.0±14.4 mmHg).
Conclusion: Fetal metabolic acidosis with UCO insults is entirely dependant upon development of lactic acidosis, without significant contribution of other metabolic acids. Despite the greater placental permeability of CO_2 as compared to O_2, fetal pCO_2 rises following repetitive UCO with the development of severe combined respiratory and metabolic acidosis. These findings suggest 1) measures of lactate may provide optimal assessment of fetal metabolic acidosis status, and 2) mixed metabolic/respiratory acidosis occurring in most cases of severely acidotic fetuses may be a result of impaired placental CO_2 clearance.



370

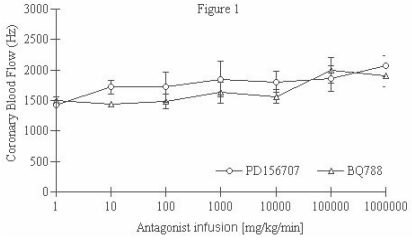
Chronic Fetal Hypoxia Results in Reduced Femoral Artery Blood Flow during Late Gestation in the Sheep. Janna L Morrison, I Caroline McMillen. (SPON: David M Olson). *Sansom Institute, University of South Australia, Adelaide, South Australia, Australia.*
Objective: The cardiovascular response to acute hypoxia has been well documented in the late gestation sheep fetus and is characterized by a fall in heart rate, a rise in blood pressure and an increase in total peripheral resistance. This results in increased blood flow to the brain, heart and adrenals and decreased blood flow to the periphery. The cardiovascular responses to chronic fetal hypoxia fetal growth restrictions are however, less well characterized.
Hypothesis: We hypothesize that there will be a reduction in blood flow to the periphery in chronically hypoxic, growth restricted fetuses in late gestation.
Methods: Uterine caruncle ctomy, to reduce placental and hence fetal growth, was performed in 15 non-pregnant sheep 10 wks prior to mating. Vascular surgery was performed at 110-120d GA with catheters implanted in the carotid and femoral artery, jugular vein and amniotic cavity and a Transonic flow probe was placed around the femoral artery. At two points in gestation (~120d and ~132d GA), blood pressure, heart rate and femoral blood flow were recorded for 2-6h (Chart, ADInstruments, Aus). Fetal arterial PO_2 was measured daily to determine if fetuses were normoxic or hypoxic (<17mmHg). Fetuses were split into 3 groups, control normoxic (n=8), PR normoxic (n=5) and PR hypoxic (n=10) and data analyzed using two way ANOVA (group & age) where P<0.05 was significant.
Results: Mean gestational PO_2 was lower in hypoxic PR fetuses (15.3±1.4mmHg) compared to normoxic control (19.8±0.5mmHg) and normoxic PR (20.6±0.8mmHg) fetuses (P=0.01). Hypoxic PR fetuses weighed less than normoxic control or PR fetuses (P=0.01). There was no difference between the three groups in mean arterial pressure, or heart rate but, femoral artery blood flow was lower at both gestational ages in PR hypoxic fetuses compared to both normoxic control and normoxic PR fetuses (P=0.01). Femoral artery blood flow increased with gestational age in all 3 groups (P=0.01). Femoral artery blood flow was directly related to mean gestational PO_2 while femoral artery resistance was inversely related to mean gestational PO_2.
Conclusions: This data supports the hypothesis that there is a redistribution of cardiac output in the chronically hypoxic, growth restricted fetus to support the growth of the brain, heart and adrenal at the expense of the periphery.

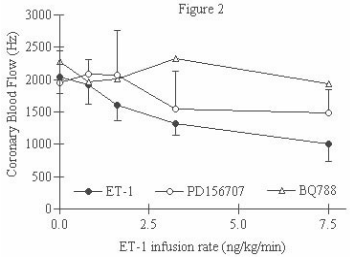
371

Endothelin-1 (ET-1) Regulates Coronary Blood Flow in Fetal Sheep. Michelle Kutzler,[*1] Sam Louey,[2] Sonnet Jonker,[2] Lowell Davis,[*2] Kent Thornburg.[*2] *[1]College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA; [2]Heart Research Center, Oregon Health Sciences University, Portland, OR, USA.*
Introduction: Regulation of the fetal coronary circulation is different from that of the adult (1). Unlike the adult heart, the coronary tree in the ovine fetus can remodel permanently during exposure to anemia (2). Endothelin-1 is a powerful vasconstrictor and may play a detrimental role in coronary development. We sought to determine the role of ET-1 in regulating coronary blood flow under basal conditions. We hypothesized that ET-1 is important in maintaining fetal coronary resting tone.
Methods: Four pregnant ewes were prepared for surgery at 128-130 dGA (term=150 dGA). Fetuses were instrumented with carotid arterial, jugular venous, right and left atrial, coronary sinus and left circumflex (coronary) arterial catheters and a left circumflex arterial Doppler flow probe. Beginning 5 days following surgery, daily coronary dose response experiments were performed. Resting coronary ET-1 tone was determined via antagonism of the type-A and type-B receptors with 10 pg - 1 µg/kg/min IV of PD156707 and BQ788, respectively. Additionally, dose responses to intracoronary ET-1 (0.8-7.5 ng/kg/min) with and without PD156707 and BQ788 were performed. Mean hemodynamic values were recorded for 60 s following the first min of each dose.
Results: Infusion of each ET-1 antagonist showed a mild increase in coronary flow (**Fig 1**). ET-1caused vasoconstriction in a dose dependent fashion that was completely blocked by BQ788 and blunted by PD156707 (**Fig 2**).

Conclusion: ET-1 regulates coronary flow under resting conditions. ET-1 is a powerful vasoconstrictor in the fetal coronary tree and is likely to be important under conditions of hemodynamic stress.

References: 1) Thornburg & Reller, 2000; 2) Wothe D et al, 2002.





372

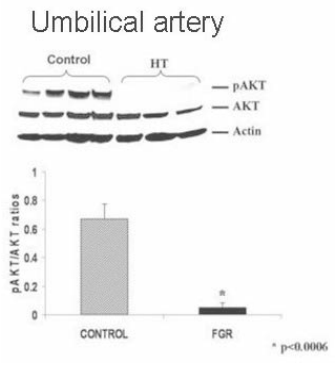
Decreased Expression of Phospho-AKT and Phospho-ERK in an Ovine Model of Intrauterine Growth Restriction (IUGR) with Systemic Hypertension. Juan A Arroyo,[1] Russel V Anthony,[2] Thomas Parker,[2] Henry L Galan.*[1] *[1]Obstetrics and Gynecology, University of Colorado and Health Sciences Center, Denver, CO, USA; [2]Pediatrics, University of Colorado and Health Sciences Center, Denver, CO, USA.*

OBJECTIVE: Both phosphorylated (p) ERK and AKT are known to be involved in endothelial cell functioning and are regulated by shear stress. In a hyperthermic (HT) ovine model of fetal growth restriction (FGR) we hypothesize that ERK and/or AKT will be phosphorylated (activated) in the umbilical and uterine vessels of 130 days gestational age (dGA) animals.

STUDY DESIGN: 4 ewes were exposed to HT conditions for 80 days to induce IUGR and 4 were placed in ambient conditions. Doppler measurements were made, and aortic catheters were placed for blood gas analysis and systemic pressure determination. Umbilical vessels and uterine artery were collected for Western blot analysis with antibodies against (p) ERK, ERK, pAKT and AKT.

RESULTS: Compared to control animals**,** HT animals demonstrated, 1) smaller fetuses (2914±201g v. 1718±433g; p≤0.03) and placentae (349±21g v. 169±22g; p≤0.03, 2) higher S/D ratios (3.0±0.34 v 3.8±0.18; p<0.009) and fetal systemic blood pressures (41±1.53 mmHg v 44.3±1.71mmHg; p<0.03), 3) decreased fetal O2 saturation (52.2±7.03% v 33.05±10.98% p<0.008) and pO2 levels (18.9±1.47 mmHg v 13.9±1.9mmHg; p<0.002), 4) a non-significant 1.8-fold decrease in umbilical vein pERK with no differences in pAKT, 5) a 14.2-fold decrease in pAKT (p<0.0006, figure) in the umbilical artery, but a non-significant 3.4-fold decrease in pERK, 6) a 4.3-fold increase in pAKT (p<0.006) in the uterine artery, but no difference pERK.

CONCLUSION: We conclude that there were non-significant differences in the umbilical vein for pERK and pAKT. However the increase pAKT in the uterine arteries will need to be explored further. In addition, pAKT was decreased in the umbilical arteries. This is opposite of what we expected due to the hypertension and hypoxia observed in these animals. This decrease observed in the umbilical arteries could be a reflection of the underlying vasculopathy characteristic of IUGR pregnancies.(Supported by NIH grant R01 HL071990-01A1).



373

Understanding Cardiac Dynamics of Low-Risk and High-Risk Fetuses by Scaling Analysis: A Magnetocardiographic (MCG) Study. Ranthinaswamy B Govindan,[1] James D Wilson,[1] Hari Eswaran,[2] Pamela Murphy,[2] Hubert Preissl,[2] Curtis L Lowery.*[2] *[1]Graduate Institute of Technology, Univ. of Arkansas at Little Rock, Little Rock, AR, USA; [2]Dept. of Obstetrics and Gynecology, Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA.*

OBJECTIVE: To study and compare the correlations using scaling analysis of the cardiac time intervals (CTI) of low-risk fetuses, fetuses at high risk for growth restriction due to placental insufficiency but with normal outcomes, and high risk fetuses with poor neonatal outcomes.

METHODS: A total of 407 fetal Magnetocardiograms (fMCG) were recorded from 99 fetuses between 27-39 weeks gestational age (GA). 265 recordings were collected from 55 high risk mothers with normal outcomes, 16 recordings from 7 high risk mothers who delivered infants with poor-outcomes, and 126 recordings from 37 low risk mothers who delivered healthy infants. After removing the maternal cardiac signals, the CTI were computed from the fMCG. The correlations in CTI were analyzed using Detrended Fluctuation Analysis (DFA) and quantified by the DFA scaling exponent 'alpha'. The difference between the groups was assessed by the student's t-test and the degree of the separation between the groups which showed significant differences was assessed by Receiver Operating Curve (ROC) analysis.

RESULTS: There was a positive trend in 'alpha' with gestational age for low-risk fetuses but no trend for the other groups. There was a significant difference between low-risk and high-risk groups between 27-31 GA which was not found in later GA range. The comparison between the poor-outcome group and the other two groups were made by including all GA because of lower number of fetuses in the poor-outcome group. There was a significant difference in between the low-risk and the poor-outcome group. and a significant difference between of the poor-outcome and high-risk group (with normal outcome). Based on the ROC analysis there was 66% separation between low-risk and high-risk groups (in 27-31 GA), 75 % separation between low-risk and poor-outcome groups and 71% separation between high-risk and poor-outcome groups.

CONCLUSIONS: The positive correlation between 'alpha' and GA for low-risk fetuses, can be explained as an indication of functional development of the autonomic nervous system (ANS). The differences between the low-risk and other two groups may be due to the early development of the ANS in the high-risk/normal outcome and high-risk/poor-outcome groups.

374

Nonlinear Components of Complexity of Fetal Heart Rate (FHR) Variability (fHRV) Specifically Reflect Sympathetic Modulation of FHR in near Term Ovine Fetus. Martin G Frasch,[1,3] Thomas Mueller,[2] Weiss Christian,[3] Harald Schubert,[2] Otto W Witte,[3] Dirk Hoyer,[3] Matthias Schwab.*[3] *[1]Ob/Gyn, Univ Western Ontario, London, ON, Canada; [2]Inst Lab Anim Sci, Friedrich Schiller Univ, Jena, Thueringia, Germany; [3]Dept Neurol, Friedrich Schiller Univ, Jena, Thueringia, Germany.*

FHRV reflects modulation of FHR by sympathetic and vagal activity of autonomic nervous system (ANS). FHRV analysis from fetal ECG is the only possibility to monitor the ANS activity non-invasively. HRV measures $aAIF_{short}$ and $aAIF_{int}$ derived from autonomic information flow (AIF) of HRV reflect communication in the ANS depending on sleep states in human neonates (Early Hum Dev, 2006). FHRV complexity depends on fetal behavioral states (Biomed Tech (Berl) 51, 2006: 233-236). While relations of fHRV linear properties to vagal and sympathetic activities were reported (J Soc Gynecol Invest 13 No. 2 (Suppl), 2006: 298A), nonlinear properties of fHRV complexity and their physiological meaning have not yet been investigated systematically.

Objective: To study how fHRV complexity measures reflect the vagal and sympathetic modulation of FHR.

Methods: FHRV was studied in fetal sheep at 127±3 days gestational age (term 150 days, n=6) at baseline in NREM and REM sleep and after vagal and sympathetic blockade with 2.5mg atropine i.v. as a 5ml bolus and, 24 h later, 2mg propranolol as a 2ml bolus over 60 sec according to Yu et al. (Pediatr Res 47, 2000:23). AIF-derived fHRV complexity measures $aAIF_{short}$ (measured over short time scale of fHRV) and $aAIF_{int}$ (measured as integral over all physiologically relevant time scales of fHRV) were assessed with and without nonlinear components using Theiler test of surrogate data to determine the linear components of fHRV complexity.

Results: $aAIF_{int}$ increased after *vagal* blockade versus baseline corresponding to decrease of fHRV complexity from 2.91±0.42 to 4.65±0.67 bit*s (p<0.05). The *nonlinear*, but not linear components of $aAIF_{short}$ increased after *sympathetic* blockade from 0.19±0.03 to 0.57±0.17 bit corresponding to fHRV complexity increase (p<0.05).

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Conclusions: While conventional fHRV measures cannot *specifically* assess the impact of reduced sympathetic activity on FHR modulation, nonlinear components of fHRV complexity derived from AIF of fHRV as presented here specifically reflect changes in sympathetic modulation of FHR. They might serve as a useful tool to detect previously unseen changes of sympathetic and vagal activities of ANS in compromised fetuses.

375

Uterine Artery Blood Flow Volume Is Reduced and Inversely Correlated to Pulsatility Index in Pregnancies with Increased Utero-Placental Impedance. Serena Rigano,[1] Simona Boito,[2] Giacarlo Pennati,[3] Alessandra Padoan,[1] Luca Mandia,[2] Erika Maspero,[1] Giorgio Pardi,[2] Enrico Ferrazzi.[1] (SPON: Felice Petraglia). *[1]Ob/Gyn, DSC L. Sacco, University of Milan, Milan, Italy; [2]Ob/Gyn, IRCCS Foundation Policlinico Mangiagalli, University of Milan, Milan, Italy; [3]LABS, Politecnico of Milan, Milan, Italy.*

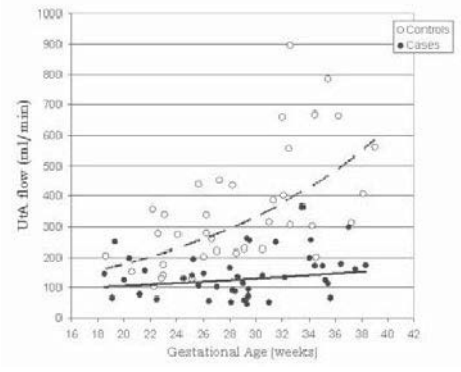
Objective: 1)to measure uterine artery (UtA) flow volume changes in pregnancies with abnormal UtA Pulsatility Index (PI); 2) to correlate UtA flow to severity of abnormal UtA PI.

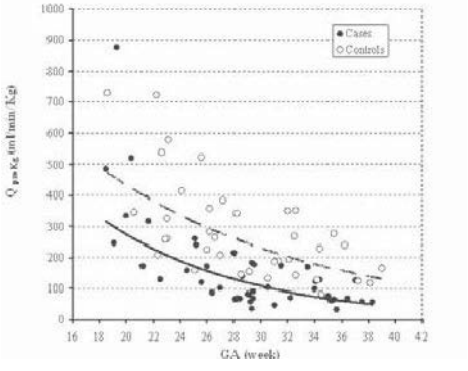
METHODS: Forty-four singleton pregnancies with abnormal UtA PI were included. Thirty-seven singleton pregnancies, normal UtA PI, were selected as age-matched controls. UtAs were identified by power-Doppler mode; after removing power-Doppler, diameter (D) was measured on a perpendicular view of UtA 10-15 millimeters prior to its division; UtA PI and time averaged peak velocity (V) were measured with an angle <30°. UtA flow (Q) was estimated by the formula $Q=hV\cdot pD^2/4$; a patient specific *h* coefficient was obtained by an *ad hoc* finite elements mathematical model.

RESULTS: Epoch at birth and birthweight were significantly lower within cases, (35±5.1 vs. 39.3±1.2 wks, p=0.0001; 2176±814 vs 3290±243 gms, p=0.002).

1) UtA flow (ml/min) was significantly lower in cases than in controls, not correlated to epoch ($R^2 = 0.0462$). A significant correlation was observed in controls ($R^2 = 0.3983$) (Figure 1). UtA flow per unit fetal weight (EFW) (ml/min/kg) was inversely correlated to epoch in cases and controls (Figure 2); 2) UtA flow was inversely correlated to UtA PI ($R^2 = 0.2855$).

CONCLUSIONS: 1) UtA flow volume (ml/min) was early and persistently reduced in pregnancies with abnormal UtA PI, compared to controls. UtA flow per unit EFW (ml/min/kg) was significantly lower in cases with than in controls. 2) UtA flow was inversely correlated to UtA PI either in cases and in controls.





376

Uterine Artery Blood Flow Volume Is Correlated to Placental Insertion Site in Pregnancies with Increased Utero-Placental Impedance. Serena Rigano,[1] Simona Boito,[2] Giancarlo Pennati,[3] Alessandra Padoan,[1] Luca Mandia,[2] Erika Maspero,[1] Giorgio Pardi,[2] Enrico Ferrazzi.[1] (SPON: Felice Petraglia). *[1]Ob/Gyn, DSC L. Sacco, University of Milan, Milan, Italy; [2]Ob/Gyn, IRCCS Foundation Policlinico Mangiagalli, University of Milan, Milan, Italy; [3]LABS, Politecnico of Milan, Milan, Italy.*

OBJECTIVE: to assess the correlation between blood flow volume of uterine arteries (UtA) and placental site in pregnancies with abnormal UtA Pulsatility Index (PI).

METHODS: Thirty-one singleton pregnancies with abnormal UtA PI and strictly lateral placental insertion were included. Sixteen singleton uneventful pregnancies, matched for gestational age, with normal UtA PI and lateral placenta were used as controls. UtAs were identified by power-Doppler mode; after removing power-Doppler signals, diameter (D) was measured on a perpendicular view of the UtA 10-15 millimeters prior to its division into corporal and cervical branch. UtA PI and time averaged peak velocity (V) were measured with an angle <30°. UtA flow (Q) was estimated by the formula $Q=hV\cdot pD2/4$; a patient specific h coefficient was obtained by an *ad hoc* finite elements mathematical model, developed according to 3D power-Doppler "casts".

RESULTS: Mean epoch in cases an controls was 28.4±5.2 vs. 28.5±4.8, respectively. Table below shows UtA flow volume in cases and controls. Flow volume was significantly reduced in cases in both uterine arteries. The ipsilateral flow was significantly higher than the controlateral artery.

CONCLUSIONS: In pregnancies with strictly lateral placental insertions a hemodynamic dominance of ispilateral UtA was observed in cases with abnormal UtA PI. This placental site effect was observed also in normal pregnancies. UtA flow and its determinants were significantly reduced in the abnormal UtA PI group.

	N	UtA flow (ml/min)	UtA Mean Velocity (cm/s)	UtA Diameter (cm)
Uterine artery ipsilateral to placental insertion site				
Cases: Abnormal UtA PI	31	90.7±46.1*	60.3±21.1	0.25±0.06
Controls: Normal UtA PI	16	232.7±152.6*	90.91±30.1	0.30±0.05
P value		<0.0001	0.0002	0.006
Uterine artery controlateral to placental insertion site				
Cases: Abnormal UtA PI	31	54.1±43.4*	44.1±18.1	0.22±0.06
Controls: Normal UtA PI	16	130.5±64.7*	68.5±29.9	0.28±0.04
P value		<0.0001	0.001	0.0002

* = Ipsilateral vs. controlateral UtA in cases and in controls, p<0.05.

377

Ontogeny of Growth-Related Genes in Ovine Sheep Heart. Seth A Reini,[1] Maureen Keller-Wood.*[2] *[1]Phys and Func Genomics, Univ of Florida, Gainesville, FL, USA; [2]Pharmacodynamics, Univ of Florida, Gainesville, FL, USA.*

Objective: We have found that increases in maternal cortisol result in increased fetal heart weight and left ventricular (LV) wall thickness, and increased AT2R mRNA relative to AT1R mRNA. This study was designed to investigate the ontogenetic changes in expression of genes implicated in cardiac enlargement in response to cortisol in the ovine fetal heart.

Methods: RNA was isolated from hearts of fetal lambs of 80, 100, 120, 130, and 145 days gestation or newborn lambs (n= 4-5). cDNA was synthesized and real-time PCR was performed. Changes in gene expression relative to expression of 18S were analyzed by ANOVA.

Results: There was a significant decrease in glucocorticoid receptor (GR) expression during gestation. There was no overall effect of gestational age on mineralocorticoid receptor (MR) mRNA, although both MR and GR mRNAs were greater at 80d than at 130d or in the newborn. 11βHSD1 mRNA was more abundant than 11βHSD2 mRNA by ~6 fold; 11βHSD2 expression did not change with fetal age, however 11βHSD1 expression was significantly greater at 80d and 145d than at 120 days. IGF-2 mRNA was ~100 fold more abundant than IGF-1 mRNA. IGF-1 did not change with gestational age, but IGF-2 and IGF-2R mRNAs were significantly reduced as gestation progressed. IGF-1R mRNA was decreased at 120 and 145d relative to 80d. There were similar levels of AT1R and AT2R mRNAs in fetal heart throughout the ages studied, and both were most abundant at 80d with decreases near term. No change was detected in angiotensinogen mRNA. ACE1 was more abundant in fetal heart than was ACE2 at all ages examined, however ACE2 was greatest

at 80d gestation, whereas ACE1 expression was markedly increased at 145 days of gestation and in the newborn lamb, so that the ACE1/ACE2 ratio was increased from 12 fold to more than 100 fold at term.
Conclusions: MR and GR are more abundantly expressed at 80d than at latter times in gestation, suggesting that the effects of increased fetal cortisol may be modulated within the developing heart. Both IGF-2 and IGF-2R gradually and significantly decrease in expression towards term starting at 120 days gestation, suggesting the pre-partum rise in cortisol levels may inhibit their expression in the heart. AT1R, AT2R, and ACE2 decrease in expression near term, whereas ACE1 significantly increases, suggesting local production of angiotensin II may be involved in the increase in LV myocyte volume and wall thickness after birth.

378

The Effect of Maternal Vitamin D Deficiency during Pregnancy in Rats on Fetal Cardiomyocyte Growth. Oksan Gezmish,[1] Kristen Bubb,[2] Marianne Tare,[2] Helena Parkington,[2] Enzo Porrello,[3] Mary J Black.[1] (SPON: Richard Harding). *[1]Anatomy & Cell Biology, Monash University, Clayton, Victoria, Australia; [2]Physiology, Monash University, Clayton, Victoria, Australia; [3]Baker Heart Research Institute, Prahran, Victoria, Australia.*
Background: The prevalence of vitamin D insufficiency in women of child-bearing age is increasing. Vitamin D is linked to cellular differentiation and there is some evidence to suggest that vitamin D deficiency leads to cardiomegaly in the fetus.
Objective: The aim of this study was to determine the effect of exposure to vitamin D deficiency from conception on cardiomyocyte number in rat offspring at postnatal day 3.
Methods: Sprague-Dawley rats were fed either a vitamin D deplete or vitamin D replete (control) diet during pregnancy and lactation. Cardiomyocyte number was determined in the fixed hearts of offspring (n = 10/group) at postnatal day 3, using an optical disector/fractionator stereological technique. In other litters, cardiomyocytes from 3 day old offspring were enzymatically isolated and the proportion of undifferentiated mononuclear cardiomyocytes and terminally differentiated binuclear cardiomyocytes was determined (n = 4 litters/group).
Results: At postnatal day 3 there was no difference in body weight, heart weight or heart weight to body weight ratio in the offspring between groups. The number of cardiomyocytes in hearts of the vitamin D deficient offspring was not different compared to controls ($7.316 \pm 0.101 \times 10^7$ cardiomyocytes and $8.206 \pm 0.128 \times 10^7$ cardiomocytes, respectively). At postnatal day 3 the number of differentiated cardiomyocytes was low in both the vitamin D deficient and control offspring (only 3% of nuclei were binucleated in both groups).
Conclusion: Vitamin D deficiency in utero does not affect the number of cardiomyocytes in the heart at postnatal day 3 in the rat. However, since the majority of the cardiomyocytes were undifferentiated at this time it is possible that cardiomyocyte number may be subsequently affected.

379

Spectral Analysis of Fetal Spontaneous Brain Activity. Rathinaswamy B Govindan,[1] Curtis L Lowery,[*2] Hubert T Preissl,[2] Pamela Murphy,[2] James D Wilson,[1] Hari Eswaran.[2] *[1]Graduate Institute of Technology, University of Arkansas at Little Rock, Little Rock, AR, USA; [2]Dept. of Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.*
OBJECTIVE: To understand and distinguish the fetal spontaneous brain activity (FS) acquired with fetal Magnetoencephalography (fMEG) from mothers which presented healthy singleton pregnancies (LR), mothers whose conditions may result in fetal growth-restriction (HR), mothers who smoke (SM), mothers with intrauterine growth restricted fetuses (IUGR) diagnosed in-utero, and HR mothers who delivered infants identified with poor outcomes post-natally. Spectral power of these fetuses was computed in four bands: δ (0.5-4 Hz), θ (4-8 Hz), α (8-13 Hz) and β (13-25 Hz) and compared between different groups.
METHODS: A total of 201 fMEG recordings of 6 min duration each were performed from 83 gravid women using SARA (SQUID Array for Reproductive Assessment). 45 recordings (21 subjects) were from LR group, 78 (25 subjects) from HR group, 55 (23 subjects) from SM group, 14 (7 subjects) from IUGR group and 9 (7 subjects) were from fetuses with clinically poor outcomes (PO). The maternal and fetal cardiac signals were attenuated by signal space projection technique. In order to capture FS the power spectrum of fMEG from all the sensors were estimated and the normalized band power power (NBP) was computed. For a given dataset, the NBP from different sensors was then averaged to get an estimate of the same.
RESULTS: Table1 shows the comparison of NBP between different groups using Student's t-test.
CONCLUSIONS: In LR, the occurrences of discontinuous patterns decrease with maturation while the continuous pattern increases with maturation. The difference in NBP of FS between different groups may be related to difference in the processes governing the occurrences of these patterns such as sleep-wave transitions, asphyxia.

Comparison of NBP using Student's t-test.
* insignificant p-values at the level of 0.1

Group	p-values obtained from Student's t-test			
	δ	θ	α	β
HR-Sick	0.094	0.691*	0.098	0.082
SM-PO	0.048	0.514*	0.062	0.054
SM-HR	0.536*	0.653*	0.766*	0.537*
LR-HR	0.0001	0.0001	0.0001	0.08
LR-SM	0.0001	0.0001	0.0001	0.02
LR-PO	0.19*	0.0001	0.23*	0.0011

380

Cortical Tissue PO_2 and Sagittal Sinus Blood Oxygenation in the Near-Term Fetal Sheep: Estimation of Cerebral Oxygenation. Takuji Tomimatsu, Jorge Pereyra Pena, Stephen J Lee, Lawrence D Longo.* *Center for Perinatal Biology, Departments of Physiology and Obstetrics & Gynecology, School of Medicine, Loma Linda University, Loma Linda, CA, USA.*
Objectives: Estimation of cerebral oxygenation is of critical importance in the immature as well as mature brain. In the clinical setting, two methods (cerebral tissue O_2 tension and cerebral venous blood gases) are available to estimate cerebral oxygenation. Cortical tissue O_2 tension (tPO_2) is a direct and accurate measurement; however, it represents oxygenation of only a fairly discrete region of the brain. Cerebral venous blood gas values represent global cerebral oxygenation; however, this estimate is indirect and extracerebral contamination cannot be excluded. Because of its clinical importance in perinatal medicine and the knowledge that cerebral oxygenation is affected by hypoxia and hypercapnia, we examined the correlation between cortical tPO_2 and sagittal sinus blood oxygenation under these conditions.
Methods: In 6 near-term fetal sheep, by use of a fluorescent O_2 probe in the cerebral cortex and the placement of a sagittal sinus catheter, we measured values of cortical tPO_2 and sagittal sinus oxyhemoglobin saturation (ss[HbO_2]) in response to isocapnic hypoxia and normoxic hypercapnia induced by having the ewe breathe N_2 plus air or CO_2 plus air, respectively.
Results: In response to isocapnic hypoxia, cortical tPO_2 and ss[HbO_2] correlated highly and was fairly linear below PaO_2 ~16 Torr. However, at higher PaO_2values (~16 to ~25 Torr), this correlation was unclear. In contrast, in response to hypercapnia these two values correlated well between $PaCO_2$ ~40 to ~60 Torr. This correlation was absent above $PaCO_2$ ~60 Torr.
Conclusions: In the fetus, the correlation between cerebral tissue O_2 tension and ss[HbO_2] was shown to be affected by either hypoxia or hypercapnia. One of the reasons seems to be the shape and the shift of the oxyhemoglobin dissociation curve. This information may be useful in estimating cerebral oxygenation, under several conditions of blood gas alternations. (Supported by USPHS HD-3807).

381

Expression of 5αReductase Enzymes in the Fetal Guinea Pig Brain and Placenta Following Intrauterine Growth Restriction. Hannah K Palliser,[1] Della M Yates,[1] David W Walker,[2] Jonathan J Hirst.[1] (SPON: Tamas Zakar). *[1]Mothers & Babies Research Centre, School of Biomedical Sciences, University of Newcastle, Newcastle, NSW, Australia; [2]Physiology, Monash University, Clayton, VIC, Australia.*
Objectives: Neurosteroids may play a significant role in the protection of the fetal brain when faced with hypoxic ischemic insults. Intrauterine growth restriction (IUGR) has been shown to alter steroidogenic pathways in the fetus, which may limit the level of neuroprotective steroids and/or their synthetic enzymes in the brain leaving these fetus vulnerable to brain injury. The aim of this study was to compare the expression of 5α reductase (5αR) enzymes in the brains and placentas of fetuses from normal pregnancies and those facing IUGR.
Methods: A guinea pig model of IUGR was produced by the surgical ablation of the branches of the uterine artery at mid-gestation. Fetal brains and placentas were collected at term (65 days) and prepared for immunoblotting. 5αR1 and 5αR2 expression in the cortex, hippocampul region and placentas of these chronically insulted fetuses (n=4) were compared to sham operated controls (n=4). Denistometry of the specific bands was normalized against actin.

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Results: Birth weights of IUGR fetuses were significantly lower than those of the sham operated controls (44.9±6.9g and 80.2±6.0g respectively; P=0.001). Fetal brain to body weight ratio was higher in the IUGR fetuses (170%; P=0.001) indicating brain sparing occurred in response to the chronic insult. There was no difference in the expression of 5αR1 in the cortex, hippocampul region or placenta between sham and IUGR fetuses. However the placenta was found to have the highest level of expression of 5αR1, followed by the cortex and then the hippocampal region (P<0.05) in both groups. 5αR2 expression was not detected in the guinea pig tissues.

Conclusions: A model of IUGR in the guinea pig was successfully established allowing the elucidation of the role of the brain and placenta in neurosteroid production. The finding that 5αR1 was strongly expressed in the guinea pig brain and placenta suggests that this isoform is responsible for neurosterid production. As 5αR1 expression did not differ between these groups, suggesting either this enzyme does not control neurosteroid production in the fetal brain or that expression was maximally induced by term. The large amount of 5αR1 in the placenta suggests it plays a more important role in neurosteroid production during fetal life than has been previously suggested.

382

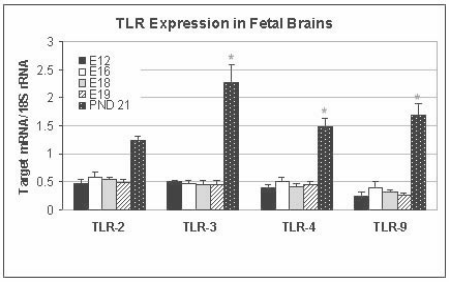
Does Gestational Age Effect Susceptibility of the Fetal Brain to an Immune Challenge? Juan Gonzalez, Jinhua Chai, Traci Lifsted, Michal A Elovitz.* *OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: Much focus has been placed on the effect of inflammation-induced preterm birth and adverse neonatal outcomes, specifically in regards to cerebral palsy (CP). However, chorioamnionitis or maternal fever at term has a stronger association for the the development of CP. We sought to assess whether the immune response in the fetal brain varied by gestational age. These studies were performed to investigate the differential expression of the innnate immune response from mid-gestation to the postnatal period.

Methods: Using timed-pregnant CD-1 mice, fetal brains were harvested from different time points in gestation: E12, E16, E18, E19 (at term, prior to delivery) and from pups on postnatal day 21 (PND 21). A minimum of 3 dams per gestational day were used with 3-9 brains for each day. Quantitative PCR was performed for Toll-like receptors (TLR) 2, 3, 4 and 9. Other essential mediators of the innate immune response were investigated including IRAK-4, MD2 and MYD88. Statistical analysis was performed using One-Way ANOVA and pairwise comparison.

Results: TLR mRNA expression is in FIGURE. TLR mRNA expression was only differentially expressed in the neonatal period with relatively constant expression during 2nd half of mouse gestation. MD-2 mRNA was not differentially expressed in prenatal or postnatal period. IRAK4 mRNA was significantly increased 2.3-fold on E19 compared to earlier in gestation (p=0.003); IRAK4 mRNA was significantly decreased in the neonatal brain compared to prenatal (p<0.001). MYD88 significantly decreased in the PND 21 brain compared to E19 (p=0.03). In contrast to TLRs, IRAK4 and MYD88 were significantly down-regulated in PND 21 brains.

Conclusions: The innate immune response is differentially regulated through gestation in the fetal brain. The interplay between TLR and accessory and adaptor proteins at term in the fetal brain may allow for a more potent immune response in the fetal brain. Further research is required to determine whether differential expression of the innate immune system plays a mechanistic role in fetal brain injury in the preterm and term human fetus.



383

Applicability of Conventional Myelin Stainings in the Fetal Brain. Iwa Antonow-Schlorke,[1] Alexandra Helgert,[1] Thomas Mueller,[2] Otto W Witte,[1] Harald Schubert,[2] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller University, Jena, Germany; [2]Inst. of Lab Animal Sciences, Friedrich Schiller University, Jena, Germany.*

Conventional histochemical myelin staining techniques with Luxol Fast Blue and phosphotungstic acid/ levanol were developed in the adult brain. The applicability of these techniques to the developing brain is questionable because of the temporary occurrence of neuronal and glial structures that are important for brain development.

Aim: Evaluation of the suitability of staining with Luxol Fast Blue and phosphotungstic acid/ levanol to label myelin in the developing brain.

Methods: Samples of ovine fetal brains were collected at 0.27 (n=6), 0.40 (n=7), 0.53 (n=7), 0.63 (n=6), 0.75 (n=6) and 0.87 (n=3) gestation (term 150 days). Brain samples were immersion fixed (0.27 and 0.53 gestation) or brains were perfusion fixed with 4% paraformaldehyde followed by paraffin embedding. 7 µm coronal sections of the forebrain including the lateral ventricles and cortical plate were cut. Luxol Fast Blue and phosphotungstic acid/ levanol stainings were compared with the immunoreactivity (IR) of myelin basic protein (MBP), a specific marker of mature oligodendrocytes, and glial fibrillary acid protein (GFAP), a marker of radial glia early in development. Hematoxylin & eosin (H&E) was used to visualize anatomy.

Results: MBP IR was detected first at 0.63 gestation indicating onset of myelination. In contrast, Luxol Fast Blue and phosphotungstic acid/ levanol revealed dense staining patterns before onset of myelination. These patterns represented radial glia but also erythrocytes and cell nuclei as known from the adult brain. As expected, Luxol Fast Blue and phosphotungstic acid/ levanol stained myelinated fibers after onset of myelination.

Conclusion: Conventional myelin staining techniques do not allow a clear discrimination of myelinated fibres in the developing brain but are useful techniques if myelination is finished or the time course of myelination of the regions of interest is well known. Otherwise the use of antibodies against MBP is recommended.

384

Accelerated Age-Related Decline in Renal Function and Altered Vascular Function in Aged Female Rats That Were Exposed to Maternal Protein Restriction *In Utero*. Mary J Black,[1] Amanda Sampson,[2] Monika Zimanyi,[1] Rebecca Flower,[2] Kristen Bubb,[2] Marianne Tare,[2] Kate Denton.[2] (SPON: Richard Harding). *[1]Anatomy & Cell Biology, Monash University, Clayton, Victoria, Australia; [2]Physiology, Monash University, Clayton, Victoria, Australia.*

Objective: Maternal protein restriction leads to low birth weight, reduced nephron endowment and altered vascular reactivity in young offspring, which may program for disease later in life. This study determined the effect of gestational dietary protein restriction on renal and vascular function in aged rats.

Methods: Pregnant rats were fed a normal (NPD; 20% casein) or low (LPD; 8.7% casein) protein diet throughout gestation. Conscious mean arterial pressure (MAP) and anaesthetised renal function were measured via clearance methods in NPD (n=8, 4 male) and LPD (n=10, 5 male) offspring at 100 weeks of age. Vascular function and mechanical wall properties in mesenteric arteries from NPD and LPD offspring were also assessed using wire and pressure myographs.

Results: At 100 weeks of age, body weight and MAP was not different between the dietary groups. Glomerular filtration rate was 0.26 ± 0.04 ml/min/gKW (NPD-male), 0.86±0.16 ml/min/gKW (NPD-female), 0.27 ± 0.04 ml/min/gKW (LPD-male) and 0.40 ± 0.13 ml/min/gKW (LPD-female), respectively (P_{DS}= 0.02; 2-way ANOVA factors diet and sex). In addition, there was reduced sensitivity to the nitric oxide donor, nitroprusside in mesenteric arteries of LPD offspring compared to NPD controls. There was a gender-dependent enhancement of wall tension generated upon pressurization of mesenteric arteries of female LPD offspring, but not in arteries from LPD male offspring when compared to NPD controls.

Conclusion: Female offspring exposed to maternal protein restriction may be at greater risk of renal failure and vascular dysfunction at old age when compared to their male counterparts.

385

TGFβ Mediated Glomerulogenesis in Offspring Exposed to Maternal Hypernatremia. Roy Z Mansano,[1] Mina Desai,[1] Darran N Tosh,[2] Ambica Garg,[1] Gyu Y Choi,[1] Michael G Ross.*[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Adelaide, Australia.*

Objective: Maternal nutrient restriction (NR) results in growth restricted newborns which exhibit hypertension, and reduced glomerular number with compensatory glomerular hypertrophy. Maternal water restriction (WR) and associated dehydration-anorexia also produces growth restricted newborns with hypertension, though a unique programmed phenotype of paradoxically increased glomerular number. As Transforming Growth Factor Beta (TGFβ) family has both stimulatory and inhibitory effects on nephrogenesis, we hypothesized that maternal NR and WR-induced morphologic renal changes are secondary to TGFβ-mediated effects.

Methods: From day 10 to term gestation, pregnant rats received either ad libitum food and water (Control, n=7), or WR to produce an increment of ~6 mEq/l in plasma sodium (n=7), or were NR (~25%) to equivalent food as that consumed by the WR group (n=7). Following delivery, all dams received ad libitum food and water. At d1 after birth, offspring kidneys were extracted for mRNA. TGFβ receptors 1 and 2 mRNA levels were determined using real-time RT-PCR (presented as fold difference normalized to GAPDH). At d21, offspring glomerular number and size were determined in formalin fixed 5μm sections using histomorphometric analysis. Values are means±SE.

Results: WR offspring had higher, whereas NR offspring had lower glomerular number than control (WR 25±1, NR 18±1, Control 22±1 per mm2, $p<0.01$). Whilst WR pups had comparable glomeruli size as controls, NR offspring had larger glomeruli (WR 2.2±0.1, NR 2.7±0.1, Control 2.2±0.1 nm2, $p<0.01$). TGFβ R1 mRNA expression was increased in WR and NR offspring kidney (WR 3.5±0.7, NR 5.5±1.1, Control 1.0±0.2, $p<0.01$) as compared to controls. In contrast, TGFβ R2 mRNA expression was increased only in NR offspring (WR 1.9±0.5, NR 5.1±0.8, Control 1.0±0.3, $p<0.05$).

Discussion: Prenatally WR and NR offspring demonstrate systemic hypertension with markedly different renal phenotypic changes. TGFβ interacts with three receptors subtypes, but must initially bind TGFβ R2 on the cell surface. Thus, increased TGFβ R2 expression suggests a putative mechanism for disruption of nephrogenesis in NR, while increased TGFβ R1 expression does not appear to impact nephrogenesis. Modulation of newborn TGFβ expression and action may represent a therapeutic option for optimization of renal function in growth restricted offspring.

386

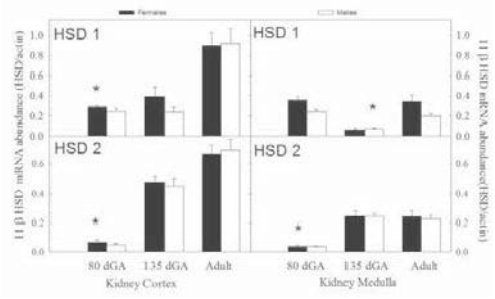
Developmental and Regional Differences in 11 β Hydroxy Steroid Dehydrogenase (11 β HSD) 1 and 2 mRNA Expression in the Sheep Kidney. Jie Zhang, Angela G Massmann, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

OBJECTIVE: The two isoforms (1 and 2) of the enzyme 11 β-HSD catalyze the interconversion of active cortisol and inactive cortisone. Little is known about the expression of these isoforms in the fetal kidney cortex and medulla. 11 β-HSD 2 is the predominant isoform in kidney where ii is thought to regulate cortisol exposure in cells expressing mineralocorticoid receptors. The aim of this study was to compare expression levels of 11 β-HSD 1 and 2 in kidney cortex (KC) and medulla (KM) in fetal and adult sheep of both sexes.

METHODS: Kidney cortex and medulla were obtained from 18 fetuses at 80 days gestational age (dGA), 16 fetuses at 135 dGA and 20 adult sheep euthanized under general anesthesia. Adult female tissue was obtained at a time of low estrogen levels in the estrous cycle. Estrus synchronization was performed by implanting sheep with EZBreed (progesterone delivery device) for 14 days. 11β HSD 1 and 2 expression was measured using Ribonuclease Protection Assay with probes developed in our laboratory. Results are expressed as Mean ±SEM and were analyzed by two way ANOVA.

RESULTS: Both 11 β-HSD 1 and 2 were present in KC and KM at all ages. A clear developmental increase in 11 β-HSD 2 mRNA expression was observed in KC and KM. In KM, the highest levels of 11 β-HSD 2 mRNA was reached by 135 dGA, whereas in the cortex adult levels are the highest. 11 β-HSD 1 expression in KC was higher in adults than in fetuses. In KM the lowest levels of 11 β-HSD 1 mRNA expression were observed at 135 dGA.

CONCLUSION: Expression of the two isozymes of 11 β HSD is developmentally regulated in kidney cortex and medulla and no sexual dimorphism is observed. Understanding of the mechanisms regulating the differential expression should allow for a better interpretation of glucocorticoids effect in the developing kidney. HL68728, P01 HD04784.



387

Ontogeny of Angiotensin Converting Enzyme 2 (ACE2), Angiotensin Converting Enzyme (ACE), and Neprilysin Activity in Fetal and Adult Sheep Kidney Cortex. Jennifer G Smith,[1] Mark C Chappell,[2] Brian M Westwood,[2] Jorge Figueroa,*[1] James C Rose.*[1] *[1]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston Salem, NC, USA; [2]Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston Salem, NC, USA.*

Background: The renin-angiotensin system (RAS) has become increasingly more complex over the past several years with the discovery of several key enzymes contributing to the metabolism of angiotensin. ACE2, a recently described homologue of ACE, metabolizes the potent vasoconstrictor angiotensin II (Ang II) to angiotensin (Ang)-(1-7) while neprilysin cleaves Ang I to Ang-(1-7) and Ang-(1-4). ACE is the major Ang II forming enzyme in the kidney, but also metabolizes Ang-(1-7) to Ang-(1-5).

Objective: To characterize the developmental profile of the enzymes responsible for angiotensin metabolism in fetal and adult sheep kidney cortex.

Methods: Solubilized kidney cortex membranes were prepared from male 80 day gestation (dg), 120 dg, 135 dg, and adult sheep. Enzyme assays were performed by incubating either ^{125}I-Ang I or ^{125}I-Ang II with the solubilized membranes in the presence or absence of lisinopril (10uM) to inhibit ACE activity, MLN4760 (10uM) to inhibit ACE2 activity, and SCH39370 (10uM) to inhibit neprilysin activity. Separation of metabolic products was achieved by reverse phase high performance liquid chromatography (RP-HPLC) and the rate of enzymatic activity quantified. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison test for post-hoc analysis.

Results: Neprilysin activity decreased throughout gestation, with lowest levels of activity seen in adult animals ($p<0.05$). ACE2 activity also decreased throughout gestation but then increased by adult life ($p=0.01$). In contrast, ACE activity tended to increase throughout gestation. The relative amounts of these peptidases also differ depending on gestational age. ACE2 and neprilysin activities are 3.5 and 4.6 times greater than ACE activity at 80 dg ($p<0.05$). Compared to ACE activity, neprilysin activity is 2.7 fold greater at 120 dg ($p<0.01$). In adults, ACE2 activity is 2.6 fold greater than ACE activity ($p<0.05$).

Conclusions: In sheep kidney cortex, the enzymes of the RAS are developmentally regulated. The developmental profile of ACE, ACE2, and neprilysin suggest that, in sheep, intrarenal metabolism of angiotensin favors the production of angiotensin-(1-7). HD 47584.

388

Fetal Sheep Unilateral Nephrectomy Alters Contractile Properties of Resistance Skin Arteries in the Adult. Victor M Pulgar, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston Salem, NC, USA.*

OBJECTIVE. Unilateral nephrectomy during fetal development in sheep results in decreased nephrons number, and in higher arterial blood pressure. While there is a decrease in GFR, the mechanisms leading to hypertension in this model are unknown. The aim of this study was to determine the effects fetal unilateral nephrectomy has on the resistance arteries response to vasoconstrictors and vasodilators.

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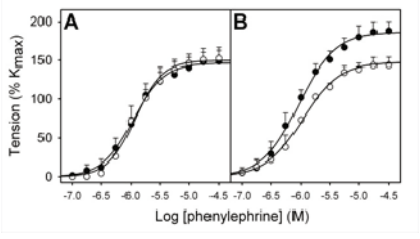
METHODS. Fetal sheep underwent left nephrectomy at 104 days of gestational age. At the adult stage (~1.5 y) a small piece of skin (~4 cm^2) was removed during surgery in nephrectomized (NX, n=4) and control (SNX, n=3) animals. Small arteries (~200 μm) were dissected and mounted in a Multi Wire Myograph (Model 610, DMT) for recording of isometric force. Dose-response curves for potassium chloride, acetylcholine, phenylephrine and sodium nitroprusside were studied alone and in the presence of indomethacin (10^{-5}M) or L-NAME (10^{-4}M). Maximal response (N/m or %Kmax) and sensitivity (pD_2) were analyzed. Data are shown as mean±SEM. Values were analyzed by unpaired *t*-test and were considered significantly different if $P<0.05$.

RESULTS. No differences were found in maximal response or sensitivity to potassium chloride (NX 6.1±0.6 *vs* SNX 7.3±1.4 N/m, $p>0.05$). Acetylcholine shows similar maximal relaxation and sensitivity in preconstricted arteries. Incubation with the α_1-adrenergic agonist phenylephrine produces a similar contractile response (Panel A) whereas pre incubation with L-NAME induces a greater maximal response in NX animals (Panel B, 189±13 %Kmax *vs* 146±4%Kmax, $P<0.05$) with similar sensitivity (pD_2 6.05±0.08 *vs* 6.09±0.08, $P>0.05$). No differences were observed in the response to sodium nitroprusside or with pre-incubation with indomethacin.

CONCLUSIONS. Skin arteries of uni-nephrectomized adult sheep show a greater nitric oxide-mediated vasorelaxant activity. This activity appears to be dependent of α_1-adrenergic stimulation. We interpret these findings as a compensatory mechanism for the higher blood pressure observed. HL 68728.



389

Localization of Inhibitory and Stimulatory Muscarinic Receptor Subtypes in Ovine Fetal Distal Colon: Implications for Meconium Passage. Jayaraman Lakshmanan, Guong L Liu, Noboru Oyachi, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Acetylcholine (Ach) mediates gastrointestinal motility through muscarinic receptors. To date five muscarinic receptors (M1 to M5) have been cloned, with M3 confirmed as the prominent mediator of smooth muscle contraction. Of other subtypes, M1, M2 and M4 have been shown to function as potent autoinhibitors (i.e. inhibitory presynaptic muscarinic receptors) of Ach release from myenteric plexus (MPL). We sought to examine the maturation and topographical distribution of muscarinic receptor subtypes in ovine fetal distal colon to assess the potential for cholinergic-induced meconium passage.

Methods: Ovine fetuses (n=4 for each gestational ages) at very preterm (VPT: 118-120 days), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (T: 146-147 days) were sacrificed and colonic segments obtained. Bouin's solution fixed, paraffin sections of distal colon were immunostained with muscarinic receptor subtype specific antibodies M1-M5 obtained from commercial sources. Immunoreactive material on the sections was identified by avidin-biotin-peroxidase system using Vectastain ABC-kit (Vector Laboratories, Inc). Immunostaining pattern was examined microscopically.

Result: M1-M5 antibodies elicited positive staining in colonic sections at all gestational ages. M4 is expressed most abundantly in circular and longitudinal smooth muscle layers while M3 expressed in rather low levels. M2 expression is more prominent in interstitial Cajal cells in the muscle and myenteric layers. Positive immunostaining for both M5 and M1 in smooth muscle layers are similar but their immunostaining intensity was significantly lower compared to M3, M4 and M2. The percentage of neurons expressing inhibitory autoreceptors (i.e., M1, M2 and M4) in the enteric ganglia was markedly higher (18 to 45%) in distal colonic sections of preterm and near term ovine fetuses, with expression declining rapidly and markedly (2%) at term ($p<0.05$).

Conclusion: These results indicate that inhibitory presynaptic muscarinic receptors in ovine fetal distal colon are developmentally regulated. We postulate that prior to term, muscarinic autoreceptors may limit presynaptic Ach release. The reduction in inhibition at term may result in cholinergically-mediated colonic motility leading to intrauterine meconium passage.

390

Maturation of Fetal Myenteric Plexus: A Physiologic Requirement for Meconium Passage. Jayaraman Lakshmanan, Guong L Liu, Michael G Ross.* *Dept. Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Meconium (MEC) passage is a developmentally programmed event occurring within the first 24-48hrs after birth. Little is known of the underlying molecular mechanisms. Delayed MEC passage observed in infants born with Hirschsprung disease provides evidence that myenteric plexuses (MPL) are required for appropriate colonic function. We hypothesize that the lack of MEC passage until birth is due to "programmed" MPL maturation. Here, we sought to delineate programmed maturation of MPL by evaluating neuronal differentiation, neuronal nitric oxide synthase (nNOS) expression, and the anatomical distribution of MPL in rat fetus on embryonic day 21 (term=22) and on the day of birth.

Methods**:** Whole gastrointestinal (GI) tract removed from rat fetuses at e21 and newborn (less than 24 hours after birth) were fixed in Bouin's solution and paraffin embedded. Sections were cut and processed for immunostaining with anti-Human neuronal protein HuC/HuD antibodies (1:800 to 1:1000) and nNOS antibodies by standard ABC regimen using Vectastain ABC kit (Vector Laboratory). The anti Hu antibodies bind specifically to antigens present exclusively in differentiated neuronal cells. The nNOS is a specific marker for inhibitory motor neurons or interneurons. Anatomical distribution of MPL was evaluated by examining whether MPL are present as a continuous belt or in regularly spaced knots as examined under microscope at 5X magnification.

Results: Of the myenteric neurons (total neurons counted:1000) 80±3% exhibited immunostaining to anti-Hu antibodies, both in e21 fetal and newborn gastrointestinal tract. The percent of neurons expressing nNOS in e21 rat fetal GI tract (19±3%; total neurons counted per fetus: 2000) was significantly greater than that in the newborn (14±2%; $p<0.05$; total neurons counted per newborn: 2300). Morphological examination revealed presence of HuD stained neurons as continuous belt in 70% of lumens at e21 as opposed to 30% of lumens in newborn ($p<0.05$).

Conclusion: These results suggest that a similar number of neurons have undergone differentiation in fetal as compared to newborn rat GI tract. The significant reduction in nNOS expressing neurons in newborn GI tract indicates that inhibitory motor neuron function is greatly diminished at birth. Based on the presence of MPL as a continuous belt in a higher proportion of e21 fetal lumens, we suggest that MPL is anatomically immature during fetal period of development.

391

S1P and LPA Regulate Surface Expression of N-Cadherin and Cell-Cell Attachment in Epithelial Ovarian Carcinoma. Yoel Smicun, Orlando Gil, Kate Devine, David A Fishman.* *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*

Objectives: Lysophophatidic acid (LPA) and sphingosine-1-phosphate (S1P) are bioactive phospholipids found in serum, ascites and the tumor microenvironment of ovarian cancer patients. We previously demonstrated that LPA and S1P regulate invasion of epithelial ovarian carcinoma (EOC) cells. Since cell-cell attachment affects motility and invasion, we investigated the effects of S1P and LPA on cell-cell attachment and related surface proteins.

Methods: After 4 hrs treatment with 40μM LPA, 0.5μM S1P or 20μM S1P, Dov13 EOC cells were labeled and applied to wells coated with a monolayer of untreated and unlabeled Dov13 cells, incubated, washed and counted. Proteins were extracted from some cells after treatment while others were detached and allowed to reattach for 3, 6 or 24 hrs (to simulate invasion) before extraction of proteins. Extracts were separated into membrane and cytoplasm fractions. Cell surface proteins of treated cells were biotinylated prior to lysis and affinity purified. Proteins were analyzed by Western blot.

Results: Dov 13 cell-cell adhesion was inhibited by half (P=0.002) upon treatment with 40μM LPA and by 26% (P=0.017) with 0.5μM S1P. A highly reproducible (though not statistically significant) 20% increase in adhesion was induced by 20μM S1P. Addition of blocking anti-integrin and anti-N-cadherin to untreated cells in the attachment assay revealed that 15% attached to ECM and 30% to monolayer cells. Membrane N-cadherin (N-cad) and γ- and β-catenins were increased by 20μM S1P and reduced by LPA in attached cells immediately post-treatment. Reattaching cells were deficient in N-cad at 3 hrs for all treatments. N-cad in 20μM S1P-treated cells recovered quickly (by 6 hrs), while treatment with 0.5μM S1P or LPA delayed N-cad recovery. γ-and β-catenins were up-regulated by 20μM S1P at 6 and 24 hrs and by 40μM LPA at 6 hrs. Cytoplasmic proteins were not significantly affected by the treatments.

Conclusions: Previously demonstrated inhibition of invasion by 20μM and stimulation by 0.5μM S1P and 40μM LPA correlate with respective elevation and reduction in cell-cell adhesion. Up- and down-regulation of the adhesion protein N-cad and its linkers, γ-and β-catenins, correspond to increased and diminished adhesion, respectively. This indicates that ovarian cancer invasion, as regulated by S1P and LPA, is associated with significant cell surface protein modifications.

392

Is There a Role for the Androgen Receptor (AR) in Ovarian Cancer Invasion? Radhika Ghatge,[*1] Marek Kudla,[1] Orlando Gill,[1] Kate Horwitz,[2] David Fishman.[*1] *[1]Department of Obstetrics and Gynecology, New York University, New York, NY, USA; [2]Department of Medicine, University of Colorado, Denver, CO, USA.*

Objectives:
Androgens play an integral role in the physiologic and pathologic processes of the ovary. Yet it has been difficult to study the role of androgen receptors (AR) separately from the other steroid receptors such as the progesterone receptor (PR)in ovarian cancer. This has been made more complicated because most synthetic progestins such as Medroxyprogesterone acetate (MPA) have promiscuous activity, binding to both PR and AR. The objectives of our study were: 1. To create an ovarian cancer cell line constitutively expressing only AR. 2. To compare the role of AR activated by the synthetic progestin MPA vs. the pure androgen dihydrotestosterone (DHT) on invasiveness of human breast and ovarian cancer cells. 3. To investigate the mechanisms of this invasiveness.

Methods:
ER- and PR- human breast (T47D-Y) and ovarian (OvCa 429) cancer cells were engineered to stably express AR. Immunocytochemistry and western blot analyses confirmed that these breast and ovarian cancer cell lines (called Y-AR and OvCa-AR respectively) are PR-, but AR+. Boyden chamber invasion assays were performed using Y-AR and OvCa-AR cells treated with either vehicle, MPA or DHT. The mechanisms of invasion were further investigated using zymographic assays.

Results:
We show normal AR translocation in both our new cell lines upon hormone treatment. AR activation by either MPA or DHT increases the invasive potential of both breast and ovarian cancer cells with MPA being significantly more effective than DHT at stimulating invasion. However, regardless of the ligand, activation of AR increases tumor cell aggressiveness. To elucidate the mechanisms of this activation in OvCa-AR cells, we used zymographic analysis. Interestingly, we find that MPA activation of AR decreases both the total level and activation of matrix metalloproteinase-9 compared to DHT and vehicle control. Thus the enhanced aggressiveness induced by MPA must occur through pathways other than extracellular matrix degradation.

Conclusion:
AR plays an important role in the pathophysiology of ovarian cancer. We hypothesize that MPA and DHT have unique downstream effects through AR and are currently using proteomic analysis to identify AR targets differentially regulated by each ligand. Blockade of downstream AR targets may provide interesting therapeutic targets in the treatment of ovarian cancer.

393

Cell Attachment Proteins Respond Differently to S1P and LPA in Epithelial Ovarian Cancer Cells Than in Normal Ovarian Surface Epithelium. Yoel Smicun, Orlando Gil, David A Fishman.[*] *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*

Objective: Lysophophatidic acid (LPA) and sphingosine-1-phosphate (S1P) are bioactive phospholipids in the tumor microenvironment that regulate survival, proliferation and motility of cells. Epithelial ovarian carcinoma (EOC) cells invade extracellular matrix (ECM) more effectively than normal ovarian surface epithelial (OSE) cells. We previously demonstrated that LPA and S1P regulate EOC invasion. Here we set out to compare surface presentation of proteins associated with cell-cell and cell-matrix attachment in EOC and OSE stimulated with S1P and LPA.

Methods: Dov13 EOC and IOSE cells were treated with either 20μM S1P or 80μM LPA, for 4 or 24 hrs. Cell surface proteins were biotinylated and affinity purified. Surface expression of cell-cell adhesion protein N-cadherin and linkers γ-, β- and α-catenins and of the cell-matrix adhesion protein β1-integrin and focal adhesion proteins focal adhesion kinase (FAK) and paxilin was detected by Western blot analysis.

Results: The basal levels of surface N-cadherin and its linkers γ-,. β- and α-catenins were significantly higher in Dov13 than IOSE cells. In Dov13 cells N-cad and β-catenin were down-regulated by LPA and up-regulated by S1P, while in IOSE cells all were up-regulated by both LPA and S1P. Cell-matrix adhesion protein β1-integrin was up-regulated by S1P but unaffected by LPA in Dov13. Neither lipid seemed to affect β1-integrin in IOSE. Focal adhesion protein paxilin was increased by S1P in Dov13 cells and by LPA in IOSE cells. Basal FAK was greater in Dov13 cells, was increased by S1P in both cell lines, and was inhibited by LPA only in Dov13 cells.

Conclusions: These results show that increased Dov13 cell invasion compared to IOSE cells corresponds to differences in surface proteins and cell-cell and cell-matrix attachment proteins. First, the basal levels of cell-cell attachment and FAK were higher in Dov13 cells than in IOSE cells. Second, the response of Dov13 cells to S1P and LPA differed from that of IOSE cells; for example, proteins associated with cell-cell attachment were down-regulated in Dov13 cells but up-regulated in IOSE cells following LPA treatment. In conclusion, the malignant nature of EOC cells is reflected both by increased invasiveness as well as by increased presentation of cell surface proteins and different responses to S1P and LPA, when compared with IOSE cells.

394

2-Methoxyestradiol (2ME) Enhances TRAIL Mediated Apoptosis in Endometrial Cancer Cells but Not in Normal Cells through an Increased Activation of Both Extrinsic and Intrinsic Apoptotic Pathways. Mauricio Cuello,[1] Sumie Kato,[1] Anil Sadarangani,[2] Gareth Owen,[2] Stanley Lipkowitz,[3] Jorge Branes,[1] David Mayerson,[1] Jorge Carvajal.[*1] *[1]Obst & Gyn, Pontificia Universidad Católica de Chile (PUC), Chile; [2]Biological Sciences, PUC, Chile; [3]Lab of Cellular & Molecular Biology, CCR, NCI/NIH, Bethesda, MD, USA.*

Uterine cancer is one of the main causes of death from gynecologic cancers. Despite using extensive surgery, radiotherapy or chemotherapy there is poor survival at advanced stages. Thus, finding new strategies of treatment constitutes a great challenge. We already demonstrated that TRAIL, a member of TNF family, induces apoptosis in ovarian cancer. In addition, we have found that 2-methoxyestradiol (2ME), an estrogen metabolite, kills cancer but not normal endometrial cells. Objectives: We investigated if TRAIL induces apoptosis in endometrial cancer and if 2ME enhances TRAIL mediated apoptosis. Furthermore, we investigated the mechanisms behind any interaction between them. Methods: Cell viability was studied by the MTS assay. Apoptosis was confirmed by DNA laddering, by FACS, in vitro caspase activity assay, and W-B of different proteins involved in the apoptotic cascade. Results: TRAIL induces cell death in endometrial cancer cell lines (established from advanced carcinomas) but not in normal cells. More interesting, TRAIL did not affect cell lines established from endometrial hyperplasia or early stage carcinomas. 2ME also induces cell death in primary cultures of advanced endometrial cancer but not in pre-malignant lesions or normal endometrium. Pretreatment with 2ME enhances TRAIL mediated apoptosis in advanced endometrial carcinomas with no effect on hyperplasic or normal endometrium. The interaction was synergistic by fractional inhibition analysis. The mechanism behind this interaction involved increase in DR5 (RT-PCR, W-B), increase in BID cleavage, increase in BCL2 phosphorylation, decrease in Akt phosphorylation, increase in caspase-8, -9, and -3 activity, and increase in cytochrome-c release. The use of a non-selective caspase inhibitor (ZVAD-fmk) completely abrogated this interaction. Use of selective caspase inhibitors (caspase-8, -9 inhibitors) only partially reverted the effect. Conclusions: These data suggest 2ME enhances TRAIL mediated apoptosis by increasing both extrinsic and intrinsic apoptotic cascades. TRAIL and 2ME, might be considered in the treatment of advanced endometrial carcinoma. (FONDECYT 1050744)

395

LPA and S1P Induce Gi-Protein Dependent Changes in Ovarian Cancer Cell MMP2 Activity and MT1-MMP Expression. Kate Devine, Yoel Smicun, Catherine Lee, David Fishman.[*] *Obstetrics/Gynecology, NYU, New York, NY.*

OBJECTIVES: Matrix metalloproteinase-2 (MMP2) plays a role in epithelial ovarian cancer (EOC) progression and metastasis. We have demonstrated that phospholipids S1P and LPA, found in high levels in the serum and ascites of ovarian cancer patients, affect MMP activity and EOC cell invasion. S1P and LPA act via G-protein coupled receptor mechanisms. We therefore examined the effects of S1P and LPA on MMP2 and its activating protease (membrane type) MT1-MMP in two EOC cell lines and explored the mechanism of these effects by blockade of Gi with pertussis toxin (PTX).

METHODS: Dov13 and OVCA429 cells were treated for 6 hrs with 1, 10, or 40μM LPA or 0.05, 0.5, 1, 5, or 20μM S1P with and without 50ng/mL PTX. Conditioned media were collected and evaluated via gel zymography, and activity was quantified via fluorometric gelatinase assay. Whole cell lysates were analyzed via Western Blot. Dov13 cells treated for 24 hrs with 20μM S1P were biotinylated, affinity purified, and analyzed.
RESULTS: Gel zymography revealed MMP2 in the media of all EOC cells regardless of treatment. Fluorometric gelatinase assay revealed increased activity induced by LPA in a dose dependent fashion (low concentration, p=0.004, high concentration p=0.012). A 2-3 fold increase in activity was induced by low S1P concentrations (p=0.049). High S1P concentrations diminished activity relative to control (p=0.001). Also in accord with these results was increased MT1-MMP in whole cell lysates of 10μM LPA-treated Dov13 cells, as demonstrated by Western blot. 1 and 5μM S1P treated cell lysates displayed down-regulation of MT1-MMP. Surface MT1-MMP was also significantly decreased in cells treated with 20μM S1P for 24hrs. Interestingly, PTX increased gelatinase activity of LPA-treated Dov13 (p=0.007) and OVCA429 cells (p=0005). PTX decreased activity in cells treated with low (0.5μM) concentrations of S1P (Dov13, OVCA429; p=0.067,0.096) and increased those with high (5μM) SIP concentrations (not statistically significant), indicating that PTX counteracted the primary effect of both S1P treatment concentrations. Western blot of OVCA429 cell lysates treated with 5μM S1P and PTX showed a corresponding upregulation of MT1-MMP relative to treatment with 5μM S1P alone.
CONCLUSIONS: We have previously shown that LPA and low concentration S1P increase EOC cell invasion, while supraphysiologic S1P inhibits. MMP2 expression and activity are here shown to correlate with our previous findings.

396

Induction of Autophagy Promotes Cell Death in Ovarian Cancer Cells. Ayesha B Alvero, Michele K Montagna, Ki Hyung Kim, Gil Mor.* *Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: The role of autophagy in cancer is controversial. Initially autophagy was thought to represent an alternative pathway for cell death. More recently it has been suggested to play a role in tumor survival. In this study we describe how the induction of autophagy by a novel isoflavone derivative, NV128, induce tumor cell death in chemoresistant ovarian cancer cells.
Methods: Eight primary cultures and two established epithelial ovarian cancer (EOC) cell lines were treated with increasing concentrations of NV128 (0.1, 1, and 10 μg/ml) with or without the pan-caspase inhibitor, Z-VAD-FMK. Cell viability was determined after 24h using the Celltiter 96 assay. Caspases- 3/7, -8, and -9 activity was measured using Caspase-Glo assay. Autophagic markers LC3-II and Beclin-1 was determined by Western blot analysis and the formation of acidic vesicles was evalauted by acridine orange staining.
Results: NV128 treatment decreased cell viability in all tested EOC cells lines in a dose-dependent manner with IC50 between 1 and 5 μg/ml. However, cell death was caspase independent as evidenced by the lack of caspases- 3/7, -8, and -9 activity and cell death could not be prevented with the caspase inhibitor Z-VAD-FMK. However, cells treated with NV128 showed characteristics of autophagy: i) morphologic appearance of vacuoles; ii) formation of acidic vesicles, iii) upregulation of the autophagic markers LC3-II and Beclin-1.
Conclusion: We demonstrate that induction of autophagy by NV128 in chemoresistant ovarian cancer cells is able to promote cell death. Our results suggest that the autophagic pathway can be an alternative target in EOC cells, especially those that are highly resistant to apoptosis.

397

Cytokine Regulation of HIF-1α in Ovarian Cancer. Cheung Wong,*[1] Phani M Garimella,[2] Theresa L Wellman,[2] Karen M Lounsbury.[2] *[1]OB/GYN, University of Vermont, Burlington, VT, USA; [2]Pharmacology, University of Vermont, Burlington, VT, USA.*
The transcription factor hypoxia inducible factor 1-α (HIF-1α) plays a vital role in the transcription of genes related to angiogenesis and cell survival. HIF-1α is induced in ovarian cancer, and correlates with induction of vascular endothelial growth factor (VEGF). Cytokines, including tumor necrosis factor-α (TNF-α), are also overexpressed in ovarian cancer. TNF-α has been linked to increased stabilization of HIF-1α, yet the mechanisms of regulation and effects on hypoxic signaling have not been defined. Here, we use immunoblot analysis in SK-OV3 human ovarian cancer cells to test the ability of cytokines to regulate HIF-1α through signaling by : nuclear factor κB (NFκB), mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K). TNF-α dose-dependently increased HIF-1α levels in normoxia, but surprisingly reduced the HIF-1α response to hypoxia. Blocking NFκB with IKK inhibitor, Bay-11-7082 reduced induction of HIF-1α in presence or absence of TNF-α, suggesting that NFκB signaling suppresses basal HIF-1α levels. However, in hypoxia, Bay-11-7082 exhibited a sigmoidal dose-response curve, reducing the hypoxic HIF-1α response at low concentrations and inducing the HIF-1α response at high concentrations. Blocking MAPK using the MEK inhibitor, U0126 or blocking PI3K using Ly294002 resulted in reduction of HIF-1α in normoxia, and enhanced the inhibitory effect of TNF-α in hypoxia. These results suggest that NFκB, MAPK and PI3K play a role in cytokine regulation of HIF-1α. Importantly, our findings suggest differences in the regulation of HIF-1α by these pathways under normoxic vs. hypoxic conditions, indicating that ovarian tumors my respond differently to cytokines depending on their oxygen status.

398

The Role of COUP-TFII in Ovarian Cancer. Shannon M Hawkins,[1] Matthew Anderson,*[1] Donna Coffey,[2] Anna Sienko.[2] *[1]Department of Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX, USA; [2]Department of Pathology, The Methodist Hospital, Houston, TX, USA.*
BACKGROUND: The metastasis of ovarian cancer depends on the creation of new blood vessels. In mouse, expression of the orphan nuclear receptor, chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), plays a critical role in normal development by specifying patterns of vascular differentiation. However, the role of COUPTFII in most human cancers, including ovarian cancer, is largely unknown. **METHODS:** RNA was isolated from ovarian cancers using TRIzol. After treating each specimen with DNAase, first strand cDNA synthesis was performed using reverse transcriptase. Real time quantitative PCR was performed using validated primers for COUP-TFII, normalizing to levels of 18s rRNA expression. COUP-TFII expression was also examined using a tissue microarray constructed from 59 ovarian cancers and 10 normal ovaries. Immunohistochemical staining for COUP-TFII was independently scored for both staining intensity (0 to 3+) and the proportion of cells expressing COUP-TFII. At least 500 cells were examined in each specimen tested. **RESULTS:** Quantitation of COUP-TFII transcripts revealed a dramatic decrease in COUP-TFII expression in ovarian cancers (n=34) to levels less than 20% of those observed in normal ovary (n=6) ($p < 0.005$). This decrease was observed regardless of tumor histology (22 papillary serous cancers, 5 endometrioid cancers, 4 clear cell cancers, 2 adult granulosa cell and 1 immature teratoma). In normal ovary, robust nuclear expression of COUP-TFII was consistently observed by immunohistochemistry in more than 75% of granulosa cells and stroma (mean staining intensity = 3+). Only minimal COUP-TFII expression was observed in the epithelium in 1 of the 10 normal ovaries examined. However, low levels of COUP-TFII expression (mean staining intensity = 1+) were observed in a significant proportion of the epithelial cells in each of the 59 ovarian cancers tested. We also found that fewer stromal cells in ovarian cancers express COUP-TFII and that COUP-TFII staining intensity in those cells was decreased when compared to specimens of normal ovary ($p< 0.05$). **CONCLUSIONS**: Our data demonstrate a coordinated change in levels of COUP-TFII expression in both the epithelia and underlying stroma of ovarian cancers. These changes potentially play an important role in the pathogenesis of this disease by regulating angiogenesis.

399

A Novel Approach To Determine Paclitaxel Resistance in Ovarian Cancer. Dan-Arin Silasi, Ayesha B Alvero, Michael Kelly, Rui Chen, Peter Schwartz,* Thomas Rutherford, Gil Mor.* *Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: Early identification of chemoresistance in patients with ovarian cancer is of utmost importance in order to provide them with the most appropriate therapy. Recently we described the expression of MyD88 in ovarian cancer cells that were resistant to the cytotoxic agent paclitaxel. In addition to chemoresistance, in MyD88 positive ovarian cancer cells, paclitaxel stimulates growth and production of proinflammatory cytokines. The objective of this study was to determine the correlation of MyD88 expression in primary and recurrent epithelial ovarian cancers with the response to carboplatin and paclitaxel combination chemotherapy.
Methods: Tumors are heterogeneous structures that contain different cell populations, thus rendering the identification of specific tumor markers difficult. Using laser capture microdissection, pure cancer cells were isolated from

ovarian malignant tumors that were obtained from 20 patients at the time of surgery. The microdissected cells were evaluated for the expression of MyD88, FasL and XIAP by western blot analysis.
Results: Protein expression was observed in samples containing as low as 500 cells. The results were correlated with the clinical course of those patients. It was evident that MyD88 expression in ovarian cancer cells accurately predicts a poor response to paclitaxel chemotherapy as shown by a short progression-free interval and overall survival.
Conclusion: We describe for the first time a molecular approach to identify paclitaxel chemoresistance. Toxicity from agents without therapeutic benefit can be avoided by identifying those patients who will not respond to a specific agent. Molecular markers will enable us to design individualized treatments and improve overall survival.

400

Induction of VEGF, VEGFR-2 and LPA3 Correlates with LPA-Induced Epithelial Ovarian Cancer (EOC) Invasion. Fengqiang Wang, Elaine Barfield, David A Fishman.* *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*
Objective: We have reported that VEGF stimulates epithelial ovarian cancer (EOC) invasion and migration through the induction of VEGFR-2. In this study, we tested the effect of lysophosphatidic acid (LPA) on VEGF121, VEGF165, and VEGFRs expression in DOV13 and other EOC cell lines. We further examined the expression of the four known LPA receptors (LPA1-4) upon LPA treatment to evaluate their potential roles in LPA-induced EOC invasion.
Methods: Real time RT-PCR was used to examine the expression levels of VEGF121, VEGF165, VEGFR1, VEGFR2, Neurophilin-1 (NRP1), NRP2, and LPA1-4 mRNA in ovarian carcinoma cell lines (DOV13, OVCA429, and R182) and normal ovarian (IOSE-29) epithelium. The expression level changes of VEGF121, VEGF165, VEGFR2 and LPA1-4 upon LPA induction are also determined by real time PCR.
Results: Among the cell lines tested, VEGF165, and VEGFR-2 showed the highest expression in IOSE-29 cells. The expression of VEGFR-2 in IOSE-29 cells was approximate 25-fold higher than DOV 13 cells, 1100-fold higher than OVCA429, and a 7-fold higher than R182 cells. Both IOSE-29 and DOV13 cells expressed higher levels of VEGF121 as compared to other cell lines, however, all cell lines expressed similar levels of VEGFR1, NRP1 and NRP2. In all ovarian carcinoma cell lines tested, LPA1 is predominantly expressed. The expression level of LPA1-4 in DOV13, R182, and OVCA429 cells are all at the following order, LPA1>LPA2>LPA3>LPA4. However, in normal ovarian epithelium, LPA1 and LPA3 expression are at the same level, both of which are greater that LPA2 and LPA4. At 0.1, 1, 10, and 20 mM, LPA significantly induced the expression of VEGF121 by 50% to ~2-fold, with LPA at 1 mM showing the maximum induction effect ($P<0.05$). LPA treatment induced VEGF165 expression in a similar manner to VEGF121. At 10-40 mM, LPA concentration dependently induced VEGFR-2 expression, without significantly affecting the expression of VEGFR1. With LPA treatment at 10 mM, LPA3 expression was significantly increased by 2-fold, without significant inducing the expression of other LPA receptor.
Conclusions: Our results show that LPA significantly induces the expression of VEGF121, VEGF165, VEGFR-2, and LPA3 in DOV13 cells. LPA may stimulate invasion and metastasis through the induction of LPA3 and VEGFR-2 mediated pathway. Whether the induction of VEGFR-2 is LPA3 dependent remains to be elucidated.

401

Phenotypic Analysis of Natural Killer Cells in Patients with Epithelial Ovarian Cancer. Jennifer A Belisle,[1] Jennifer AA Gubbels,[1] Martine Migneault,[2] Claudine Rancourt,[2] Ellen Hartenbach,[1] David Kushner,[1] Joseph Connor,[1] Manish S Patankar.[1] (SPON: Ronald R Magness). *[1]Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA; [2]Department of Microbiology and Infectiology, Universite de Sherbrooke, Sherbrooke, Sherbrooke, QC, Canada.*
Objectives: The ovarian cancer marker MUC16 (CA125) inhibits human natural killer (NK) cell function and alters the expression of activating receptors on this cell type. The purpose of this study is to determine if similar phenotypic differences are also observed in NK cells derived from the peripheral blood (PB) and peritoneal fluid (PF) of patients with epithelial ovarian cancer (EOC).
Methods: Lymphocytes from the PB (PBL) and PF (PFL) were isolated from patients with advanced stage EOC. The cells were labeled with a panel of antibodies against human CD3, CD16, CD45, CD56, CD94/NKG2A, NKG2D, NKp46, NKp44, and CD158. The labeled cells were analyzed by multi-color flow cytometry. The expression of the cell surface markers was analyzed using FlowJo (Treestar) software. The MUC16 levels in the PB and PF were determined by the clinical assay for CA125. The expression of MUC16 on the PBL and PFL was monitored by flow cytometry and RT-PCR.
Results: We report a reduced expression of CD16 on the PFL of patients with EOC as compared to PBL of the same patients. Downregulation also occurs with NKp46, which is an activating receptor found on NK cells. Distinct subsets of NK cells could also be identified based on their CD56 expression. RT-PCR studies indicate that the PBL do not express MUC16 but have the capacity to capture this mucin on their cell surface.
Conclusions: Increased downregulation of CD16 and NKp46 in the PFL compared to the PBL of EOC patients was observed. In *in vitro* experiments we have shown that MUC16 causes downregulation of CD16 on healthy donor derived PBL. The increased downregulation of the NK cell activation receptors on the PFL compared to the PBL is congruent with the 10-20-fold higher concentrations of MUC16 in the PF as compared to the serum of EOC patients. This observation suggests a direct role for MUC16 in modulating NK cell responses. The immunoregulatory function of MUC16 coupled with our recent observation demonstrating its role in the peritoneal metastasis of ovarian tumors makes this mucin an important target for future anti-tumor therapies.

402

Splitting the Family: Characterization of Two Types of Epithelial Ovarian Cancer Cells. Rui Chen, AB Alvero, Dan Silasi, Michael Kelly, Irene Visintin, Gil Mor.* *Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, USA.*
Introduction. Inflammation is associated with cancer progression and chemoresistance. We hypothesized that cancer cells may also contribute to the proinflammatory microenvironment at the tumor site. We have identified two distinct subtypes of epithelial ovarian cancer (EOC) cells according to their characteristics in NF-κB activation and cytokine production that correlate to tumor survival, progression and chemo-response.
Materials. Eleven human EOC cell lines were established from primary cultures isolated from malignant ovarian cancer ascites. NF-κB activity was determined by luciferase assay using a plasmid construct containing 2 κB-binding sites before luciferase reporter gene. Expression of MyD88, IκBα, IKKα and IKKβ were determined by western blotting. Cytokine profiling was done using the Luminex 200 system. Cell viability was determined by Celltiter 96 assay, and apoptosis was measured in terms of Caspase- 3/7 activity using Caspase-Glo assay.
Results. Two different subtypes of ovarian cancer cells were identified. Type I are characterized by: i) constitutive cyclicity of NF-κB activation, ii) continue production of cytokines which increased by LPS, paclitaxel or TNF-α stimulation; iii) expression of MyD88, low IκBα, and a high IKKβ/IKKα ratio; iv) chemoresistance. Type II are characterized by; i) absence of NF-κB activity, ii) no cytokines production, iii) MyD88 negative and high IκBα expression, and a low IKKβ/IKKα ratio; iv) chemosensitive.
Conclusion. We describe for the first time, specific characteristics of chemoresistant ovarian cancer cells. Type I EOC cancer cells present a profile that promotes tumor growth, inhibits apoptosis and presented enhanced growth in the presence of paclitaxel and TNFα. In contrast, Type II EOC cells lack all these factors, further suggesting the importance of these characteristics for tumor transformation. Identification of these markers in patients' tumor samples may facilitate the adequate selection of treatment.

403

Probing Tissue Stiffness with Atomic Force Microscopy for Early Detection of Ovarian Cancer. Liahoa Chen,[1] Jacob Rotmensch.*[2] *[1]Nanotechnology, Argonne National Laboratory, Chicago, IL, USA; [2]Gynecologic Oncology, Rush Univesity Medical Center, Chicago, IL, USA.*
It has been well established that tumor tissues are stiffer than normal tissues. This is the basis of common breast self-examination in which identifying a stiff lump in breast tissue is critical for early tumor warning. While fingers can exam the macroscopic stiffness of already formed tumor tissues, measuring the precise stiffness of a tissue in microscopic scale will indicate the early transition process from normal tissue to tumor or cancer. Collaborating with Argonne national laboratory and Illinois Institute of Technology, we developed a novel ovarian cancer diagnostic method by quantifying the disparity of tissue stiffness in micro/nano scale for ovarian normal tissue and tumor tissue. By using atomic force microscopy, we quantified the stiffness of fresh benign and carcinoma ovarian tissues. The force volume image of ovarian tumor tissue showed much low (soft) and high (stiff) slop values of force spectra, indicating

a heterogeneous stiffness feature. On the contrary, normal tissues demonstrated more homogenous stiffness distribution with the Young's modulus in the narrow range of 2.2 kPa to 12.7 kPa. High-precision stiffness measurements of ovarian tissues are also expected to give insight into the structure of tissues, tumor stage, and metastases. Moreover, such measurements are of great practical value in order to quantify the effect of drugs and cancer therapy. While most of current ovarian cancer diagnostic methods are depending on the detection of cancer biomarkers, the most important advantage of the this research is that cancer cell itself will be directly detected without any involvement of biomarkers or antibodies. It opens an entirely new avenue for early cancer diagnosis in a completely novel way.

404

The Proto-Oncoprotein AKAP-Brx Regulates Proliferation of Ovarian Cancer Cells and Mediates LPA Action. Chantal M Mayers,[2] Domenica M Rubino,[3] Kerri L Marquard,[4] Marcy F Maguire,[4] James H Segars,[*1] Paul H Driggers.[1] *[1]Reproductive Biology and Medicine Branch, NICHD/NIH, Bethesda, MD, USA; [2]George Washington University Weight Management Program, Washington, DC, USA; [3]Obstetrics and Gynecology, Tufts NEMC, Boston, MA, USA.*

Brx, a member of the Dbl family of proto-oncoproteins that activate RhoA is expressed in human reproductive tissues. In ovary, Brx expression is high in corpus luteum (CL) but weak in germinal epithelium. Brx protein expression was detected in 20 samples of various low malignant potential tumors and was relatively high in some neoplasms. Since Brx is expressed in LMP ovarian tumors and is a proto-oncoprotein, we propose that Brx functions in oncogenic pathways in ovarian tumor cells.

Objective: To investigate the function of Brx in growth stimulatory pathways in ovarian cancer cells.

Results:

Sections from 125 ovarian tumors in two tumor microarrays were evaluated for Brx expression by immunohistochemical staining and light microscopy. Brx staining was observed in the majority of types and grades of ovarian tumors.

Transfected NIH3T3 cells over-expressing Brx formed tumors after injection into nude mice.

Transfection of an AKAP-Brx expression vector stimulated proliferation of OVCAR-3 (human ovarian adenocarcinoma) cells in a dose-dependent manner. Transfection of mutAKAP-Brx that cannot activate RhoA inhibited proliferation.

AKAP-Brx is involved in LPA signaling in OVCAR-3 cells. Lysophosphatidic acid (LPA) enhances proliferation, migration, and invasiveness of ovarian cancer cells. LPA receptors are G-protein coupled receptors that signal to RhoA through Ga_{12}-dependent pathways. Ga_{12} binding stimulates RhoA activation by AKAP-Brx. LPA treatment induces activation of serum response element (SRE)-dependent gene transcription through a RhoA-dependent pathway. Expression of mutAKAP-Brx inhibited basal and LPA-induced activity of the SRE. LPA treatment also induced phosphorylation of CRE binding protein (CREB) and activated the cyclic AMP response element (CRE) in OVCAR-3. mutAKAP-Brx expression inhibited basal and LPA-induced activity of the CRE.

Conclusions: Brx is expressed in various types and stages of ovarian tumors. AKAP-Brx is expressed in OVCAR-3 cells and affects proliferation by activation of RhoA. Over-expression of AKAP-Brx can drive proliferation of ovarian cancer cells. AKAP-Brx function is required for LPA signaling in ovarian cancer cells. CREB may be a LPA effector in ovarian cancer cells.

405

Effect of Hypoxia on IL-1α and Cortisol Induced Gene Expression in Sheep Ovarian Surface Epithelial Cells. Christopher R Harlow, Mick T Rae, Deborah Price, Hilary OD Critchley,[*] Stephen G Hillier. *Centre for Reprodutive Biology, University of Edinburgh, Edinburgh, United Kingdom.*

Introduction The intraperitoneal environment of the ovarian surface epithelium (OSE) has an oxygen (O_2) tension of 5-12%. Ovarian cancers arising from the OSE (~80-90% of ovarian malignancies) are likely to experience increased hypoxia due to the relatively poor vascularisation of solid tumours.

Hypothesis Hypoxia may modulate inflammatory and anti-inflammatory induced gene exression in OSE

Methods Using modular incubators gassed at 1% or 5% O_2 and a standard CO_2 incubator (20% O_2), we measured the expression of Hif-1α, HSD11B1 and COX-2 mRNA by Taqman qRT PCR in response to inflammatory (0.5ng/ml Il-1α) and anti-inflammatory (1μM cortisol (F) challenge in a model system using sheep (s) OSE over 48h at these three O_2 tensions.

Results Hif-1a binding is classically up regulated by hypoxia and by inflammatory stimuli. In sOSE, F alone had no effect on Hif-1α, whereas IL-1α increased Hif-1α 7- to 9-fold, although there was no effect of hypoxia.However, whilst F partially reversed the IL-1α effect at 20% O_2, this effect was absent at lower O_2 concentrations. In human (h) OSE, HSD11B1 mRNA is up regulated by IL-1α and this effect is enhanced by addition of F. In our sheep model we found IL-1α to have a similar stimulatory effect, and the stimulatory effect was dose-dependently increased by decreasing O_2. In contrast to hOSE, addition of F reversed the effect of IL-1α in sOSE. COX-2 mRNA followed a similar pattern to HSD11B1 in sOSE, with hypoxia again enhancing the stimulatory effect of IL-1α. In sOSE, F limits its own production in a negative feedback effect, even under severely hypoxic conditions in which the inflammatory response to IL-1α is increased. Despite hypoxia enhancing IL-1α-stimulated COX-2 mRNA expression, F is able to reverse this effect even at 1% O_2.

Conclusions The lack of effect of hypoxia on Hif-1α may reflect the time point (48h) used and further time course studies are underway. These results confirm that the hypoxic environment of the peritoneum alters gene expression in response to inflammatory stimuli and suggests that normoxia may not be an appropriate environment in which to study OSE function. Further studies of OSE and ovarian cancer cells under hypoxic conditions will permit a better understanding of the mechanisms regulating gene expression in these cells and may give insight into more effective treatment of ovarian cancer.

406

LPA Targets PAR1 To Promote EOC Invasion through the Induction of MMP-1. Fengqiang Wang, Jessica Fisher, Yelena Shutersman, David A Fishman.[*] *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*

Objectives: Lysophophatidic acid (LPA) stimulates epithelial ovarian cancer (EOC) invasion through multiple regulation pathways. Recently, protease activated receptor 1 (PAR1) was identified as a MMP-1 receptor promoting invasion in breast cancer cells. In EOC cells, we have shown the positive correlation between MMP-1 and PAR1 co-expression and cell invasiveness. In this study, we aimed to investigate whether the newly identified MMP-1-PAR1 axis plays an active role in LPA-induced EOC invasion.

Methods: Induction of MMP-1 and PAR1 expression by LPA treatment in EOC cells (DOV13 and R182) was tested by real time RT-PCR**.** The effect of LPA on PAR1 protein expression was examined by Western blotting. Cell invasion was determined by the *in vitro* Matrigel invasion assay. The anti-MMP-1 monoclonal antibody or the MMP-1 small interference RNA (siRNA) were used to down-regulate MMP-1 activity. ELISA was used to measure the total or active MMP-1 in EOC cell conditioned medium. PAR1 siRNA was transfected to DOV13 cells to knock down PAR1 expression and evaluate the role of PAR1 in LPA-induced EOC invasion. The knocking down efficiency of MMP-1 and PAR1 by siRNA was also examined by real time RT-PCR 48 hour after transfection.

Results: LPA (20 mM) treatment significantly increased mRNA expression of MMP-1 by 2 fold, and PAR1 by approximately 50% (P<0.05). LPA also considerably promoted the secretion of MMP-1 in DOV13 conditioned medium and stimulated PAR1 protein expression. Recombinant MMP-1 and serum free conditioned medium (SFCM) collected from DOV13 and R182 cells containing high concentration of MMP-1 all significantly promoted DOV13 invasion (P<0.05), with SFCM that contains more MMP-1 inducing more invasion. DOV13 and R182 SFCM-induced DOV13 invasion was significantly decreased by the addition of an anti-MMP-1 antibody in the SFCM. MMP-1-induced invasion was also inhibited by transfection of DOV13 cells with PAR1 siRNA (siPAR1-85-30), suggesting PAR1 is essential to mediate MMP-1 induced EOC invasion. Moreover, silencing of MMP-1 or PAR1 both significantly decreased LPA-induced DOV13 cells invasion (P<0.05).

Conclusions: Our results suggest that MMP-1-PAR1 axis plays an active role in the regulation of LPA-induced EOC invasion and this may represent a new mechanism by which LPA promotes EOC metastasis. Our study also identifies MMP-1 and PAR1 as potential novel targets for EOC metastasis.

407

An Imbalance of Adipocyte-Derived Angiogenic Mediators, VEGF & PEDF, Promotes Pro-Angiogenic Phenotype. Beth A Plunkett,[1] Matthew Maurice,[2] Jennifer A Doll,[2] Mona Cornwell,[2] Susan E Crawford.[2] (SPON: Serdar E Bulun). *[1]Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [2]Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.*

Hypothesis: Obesity is an independent risk factor for hormonally regulated invasive cancers; however, the mechanisms underlying this heightened risk are unclear. Angiogenesis, the development of new vessels, is essential for the growth and survival of tumors. VEGF is an angiogenic promoter secreted by many tumors. Pigment epithelium-derived factor (PEDF), an estrogen-sensitive soluble glycoprotein, is a potent inhibitor of angiogenesis. Both mediators are critical in adipocyte differentiation. We postulated that adipocytes, a rich source of angiogenic mediators, can promote a pro-angiogenic microenvironment due to a loss of PEDF.

Methods: Serum-free conditioned medium was collected from adipose tissue harvested from adult wild type C57Bl6 mice and tested in an angiogenesis migration assay using antibodies directed against VEGF and PEDF. A model of adipocyte differentiation was used whereby 3T3-L1 pre-adipocytes were treated with a well-established adipogenic cocktail and conditioned medium collected daily for eight days. PEDF protein levels were analyzed using Western blot. Adipose tissue from wild type and PEDF knockout animals was immunostained with anti-CD31 antibody and microvascular density, a hallmark of angiogenesis, calculated in five non-overlapping high power fields.

Results: Adipocytes secrete both VEGF and PEDF and media conditioned by these cells have pro-angiogenic activity due to increased levels of VEGF. More robust angiogenic activity was demonstrated when medium was pre-treated with anti-PEDF antibody suggesting that PEDF has functional inhibitory activity in adipocyte secretions. The adipocyte differentiation assay revealed reciprocal expression levels of PEDF and VEGF. High levels of PEDF were demonstrated at days 1-2 whereas PEDF was undetectable in secretions from differentiated adipocytes. In contrast, VEGF levels were highest at later time points. In vivo, adipose tissue harvested from PEDF null mice had a three fold increase in microvascular density.

Conclusion: A loss of PEDF induces a more angiogenic phenotype in differentiated adipose tissue in vitro and in vivo. These data suggest that the adipocyte population has the potential to promote a pro-tumoral microenvironment.

408

Impact of Surgical Environment on Post-Operative Peritoneal Dissemination in a Syngenic Mouse Ovarian Cancer Model. Sachiko Matsuzaki,[1,2] Michel Canis,[1,2] Nicolas Bourdel,[1,2] Claude Darcha,[3] Jean-Luc Pouly,[1,2] Gerard Mage.[1,2] (SPON: Kunihiro Okamura). *[1]CENTI, Université d'Auvergne – Clermont I, Clermont-Ferrand, France; [2]Department of Gynecology, CHU Clermont-Ferrand, Clermont-Ferrand, France; [3]Department of Pathology, CHU Clermont-Ferrand, Clermont-Ferrand, France.*

Objective: Surgery is the gold standard for the treatment of ovarian cancer. However, surgical treatment might result in the development of peritoneal dissemination partly because of the favorable peritoneal surgical environment for cell implantation. It remains unclear which mechanisms stimulate post-operative dissemination. We investigated the impact of peritoneal surgical environment on expression levels of genes involved in ovarian cancer cell adhesion, proliferation, migration and invasion over time course in a syngenic mouse model.

Study design: Adult, female C57BL6 mice were divided into four groups: anesthesia alone, CO2 pneumoperitoneum at low (2mmHg) or high (8mmHg) intraperitoneal pressure and laparotomy. A mouse ovarian cancer cell line (ID8) was injected intraperitoneally just before surgical procedures. Groups was further sub-divided into two groups of 8 animals each and a laparotomy was performed to evaluate dissemination on post-operative day (POD) 14 or 42. Disseminated nodules were collected and real-time RT-PCR was performed to investigate expression levels of beta 1 integrin, cMet, protein kinase C (PKC) beta, urokinase plasminogen activator (uPA), uPA receptor (uPAR) and plasminogen activator inhibitor-1 (PAI-1) mRNA. Comparisons were made using the one-way ANOVA. Statistical significance was defined as $P<0.05$.

Results: On POD 14: Expression levels of beta 1 integrin, cMet, PKC beta, uPA, uPAR and PAI-1 mRNA were significantly higher in the laparotomy group than the remaining three groups. There was no significant difference in expression levels of these genes among the remaining three groups. On POD 42: Expression levels of the genes examined in the laparotomy group were significantly decreased compared to those on POD14. There was no significant difference in expression levels of the genes examined among the four groups.

Conclusion: The present study suggested that surgical peritoneal environment of laparotomy (host environment) could promote expression levels of genes involved in ovarian cancer cell adhesion, proliferation, migration and invasion. This model will help to elucidate the molecular mechanisms of post-operative peritoneal dissemination to develop new therapeutic strategies.

409

Lysophosphatidic Acid (LPA) Regulates Cell Surface Expression of E-Cadherin in Ovarian Carcinoma Cells. Catherine J Lee, Edgardo V Ariztia, Phil Smith, Orlando D Gil, David A Fishman.* *Obstetrics and Gynecology, New York University School of Medicine, New York City, NY, USA.*

Objective: Lysophosphatidic acid (LPA) stimulates ovarian cancer cell invasion. E-cadherin is a calcium-dependent cell adhesion molecule that is involved in the formation and maintenance of adherens junctions of epithelial cells. Down-regulation of E-cadherin is found during metastatic progression. Here, we evaluate the regulatory roles of LPA on surface-expressed E-cadherin *in vitro*.

Methods: Ovarian carcinoma cells OVCA429 were stimulated with LPA (10uM) for 1, 4, and 24 hours and expression of E-cadherin, uPA, and uPA Receptor(uPAR) was analyzed by quantitative RT-PCR. Cell surface expression of E-cadherin at 24 hours with 10uM LPA was examined by immunofluorescence staining. Shedding of E-cadherin was analyzed by Western blot on conditioned medium from cells stimulated with LPA (0.1uM, 1.0uM, 10uM) for 4 and 24 hours. Western blot was also used to analyze surface-expressed E-cadherin and uPAR. uPA inhibitor (2.5uM) was added to the cells to inhibit the uPA proteolytic cascade. A colorimetric assay for urokinase type plasminogen activator (uPA) analysis was used to evaluate the proteinase activity of uPA in the conditioned medium of LPA treated cells.

Results: LPA increased the mRNA expression of E-cadherin and uPA, while decreasing the expression of uPAR over a 24 hour period. Immunofluorescence staining revealed down-regulation of cell surface E-cadherin expression in LPA treated cells. Western blot analysis on conditioned medium from LPA treated cells revealed the release of a 80 kDa soluble E-cadherin fragment at 24 hours but not 4 hours. Down-regulation of the 130 kDa full-length and generation of a 37 kDa C-terminal E-cadherin fragment in LPA treated cells occurred by 24 hours. Increasing concentrations of LPA were shown to increase uPA activity at 4 hours and decreased activity at 24 hours. Inhibition of uPA activity with the blocking antibody resulted in a 50% depletion of enzyme activity at both 4 and 24 hours and abrogated the effects of LPA on the extracellular cleavage of E-cadherin.

Conclusions: Our results show that LPA stimulates uPA activity and addition of the uPA inhibitor decreases the effects of LPA on E-cadherin shedding. These results suggest that the uPA proteolytic cascade is involved in E-cadherin cleavage and is activated by LPA. This process may contribute to decreased cell-cell adhesion and facilitate ovarian cancer cell invasion.

410

CD44 and Osteopontin Are Shed as a Complex from the Surface Ovarian Cancer Cells and Modulate the Invasive Phenotype. Edgardo V Ariztia, David A Fishman.* *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*

Objective: Osteopontin has been proposed as a possible marker for Epithelial Ovarian Carcinoma (EOC). However, mechanisms describing specific roles of osteopontin during ovarian cancer progression have yet to be reported. Our aim was to identify possible roles of osteopontin during invasion.

Methods: Expression of osteopontin in DOV13 cells was followed by RT-PCR. DOV13 cells treated with LPA or membrane-derived vesicles to induce invasion. Addition of an antibody against osteopontin tested its role in invasion. Osteopontin and CD44 expression were followed by immunoprecipitation, western blot analysis and immunofluorescence. Over-expression of osteopontin and down-regulation of expression (siRNA) were used to assess osteopontin's and CD44's roles during invasion.

Results: DOV 13 cells express full-length osteopontin known as variant a. During membrane vesicle shedding, osteopontin is released to the media associated with the membrane vesicles (at 4 hr). No osteopontin is detected in the vesicle supernatant. Vesicle induced and LPA induced invasion of DOV13 cells can be significantly inhibited with the addition of an anti-osteopontin antibody. Immunofluorescence shows that CD44 and osteopontin co-localize on

the cell surface. At 24 hr stimulation with LPA there is accumulation of a soluble CD44-Osteopontin complex in the media. Downregulation of osteopontin expression in DOV13 cells also affects matrigel invasion.

Conclusions: Ovarian cancer cells secrete osteopontin into the tumor microenvironment primarily associated with membrane vesicles and not in soluble form. Soluble osteopontin appears only as part of a complex with CD44. Inhibition of vesicle induced invasion by antibodies against osteopontin supports the hypothesis that osteopontin is an important modulator of membrane vesicle function and ovarian cancer invasion. The specific roles of the complex are part of our ongoing research efforts.

411

Proteomic Characterization of Ovarian Cancer Membrane-Derived Vesicles Induced with Lysophosphatidic Acid. Edgardo V Ariztia,[1] Jose L Luque-Garcia,[2] Chongfeng Xu,[2] Thomas A Neubert,[2] David A Fishman.*[1] *[1]Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA; [2]Pharmacology, New York University School of Medicine, New York, NY, USA.*

Objectives: Lysophosphatidic Acid (LPA) is a pleiotropic bioactive phospholipid that induces proliferation, reorganization of the cytoskeleton, invasion and shedding of membrane vesicles in ovarian cancer cells. These vesicles promote the invasive behavior of ovarian cancer cells, and they can also induce motility in other cellular components of the tumor microenvironment. The objective of this study is to identify and quantify specific proteins that are structural or functional components of these LPA-induced vesicles.

Methods: A variation of SILAC (Stable Isotope Labeling with Amino acids in Cell culture) was used. Epithelial ovarian carcinoma DOV13 cells were labeled for five passages in the presence of ^{13}C-Arginine and ^{13}C-Lysine. DOV13 cells were stimulated with 80 mM LPA for 4hrs in the presence of 10% fetal bovine serum (FBS) to induce the release of vesicles. 10% FBS-induced vesicles were used as control. Two sets of samples were generated. One set was non-labeled FBS-vesicles and ^{13}C-labeled FBS-LPA vesicles; the other set was the inverse. Membrane vesicles were isolated from culture media by differential centrifugation. Equal amounts of samples were combined and separated electrophoretically. Selected bands were subjected to mass spectrometry analysis by LC/ESI-MS-MS. Validation of data has included western blot analysis and Immnocytochemistry.

Results: Analysis of vesicle content by mass spectrometry has revealed that 10% FBS-vesicles and LPA-vesicles shared a number of proteins. However, LPA induced vesicles contain a separate set of proteins that are not present in the FBS-vesicles. Our first interest has turned to actin and other cytoskeletal components.

Conclusions: LPA is known to induce a variety of responses in ovarian cancer cells. Preliminary results of proteomic analysis have shown that a specific component of LPA-induced vesicles is actin. This cytoskeletal protein does not appear to be incorporated to vesicles generated with serum alone. Immunocytochemical analysis of LPA-stimulated ovarian cancer cells shows that these cells rearrange their actin cytoskeleton and form peripheral bundles close to the cell surface at the same time that vesicle shedding is occurring. Complete validation of results is a current effort.

412

Interferon Regulatory Factor-β Mediates Progesterone Action in Ovarian Cancer. Amy Hakim,[1,3] Zhihong Lin,[1] Scott Reierstad,[1] Gustavo Rodriguez,[2] Ping Yin,[1] John Lurain,[3] Julian Schink,[3] Serdar Bulun.*[1] *[1]Division of Reproductive Biology Research, Northwestern University, Chicago, IL, USA; [2]Division of Gynecologic Oncology, ENH, Evanston, IL, USA; [3]Division of Gynecologic Oncology, Northwestern University, Chicago, IL, USA.*

Objective: Although much is known about the molecular biology of progesterone action in hormonal responsive tumors such as breast and endometrial cancer, relatively little is known about the regulation of progesterone action and the role of the progesterone receptor (PR) mediated pathways in ovarian cancer. Epidemiologic and laboratory evidence suggests that progesterone action is protective against development of ovarian cancer, through regulation of growth. Our goal is to identify the direct target genes of PR in ovarian cancer cells in order to help understand the protective effects of progestins on ovarian cancer risk.

Methods: We identified genome-wide targets of PR using a PR-antibody, chromatin immunoprecipitation-PCR (ChIP) followed by cloning and sequencing of PR binding sites in the genome of the PR-positive BG-1 human ovarian cancer cell line treated for 2 hours with progesterone (10^{-6}M). The genes within the vicinity of up to 166 kilobases of the PR binding sites were tested for responsiveness to progesterone by real-time PCR. Transcripts of selected genes were knocked down by siRNA. Proliferation was evaluated by flow cytometry and BrdU incorporation assay.

Results: One hundred and fifty clones in the range of 200-700 bps were identified and blasted against the human genome. Six of these clones were mapped to the human genome and found to be within various distances from a gene: 5'-1.2 kb (C21ORF29), 16.1 kb (LOC338797) and 92.2 kb (SFRS8); 3'-0.9 kb (KRTAP10-6) and 166 kb (IRF-8); and exon 6/intron 6 (SEPN1). Interferon regulatory factor-8 (IRF-8), a known transcription factor, was chosen for further evaluation, since treatment of BG-1 cells with progesterone significantly downregulated its mRNA levels at 2 and 3 hours. Reduction of IRF-8 expression by 75% employing RNA interference decreased proliferation (S-phase and G_2M) significantly in BG-1 cells.

Conclusion: Genome-wide ChIP cloning was used to identify novel PR targets in an ovarian cancer cell line in an attempt to understand the protective mechanism of progesterone action. The transcription factor IRF-8 is regulated by progesterone and may mediate its anti-proliferative effects in ovarian cancer cells.

413

Effect of Raloxifene on the 3-Year Risk of New Vertebral Fractures in Women with Low or High Calculated 5-Year Risks of Invasive Breast Cancer. Mayme Wong, Adeline A Yeo, Yongming Qu, John L Stock, Beth Mitchell, John L Mershon. (SPON: Kelle H Moley). *US Women's Health and Reproductive Medicine, Eli Lilly and Company, Indianapolis, IN, USA.*

Background: Some studies have found bone mineral density to be related to breast cancer (BC) risk, but that between BC risk and new vertebral fracture (VF) is less well studied. Raloxifene (RLX) decreases VF risk.

Methods: We examined the effects of RLX on new VF risk in 4011 postmenopausal women, grouped by calculated BC risk, who chose to enroll in the Continuing Outcomes Relevant to Evista (CORE) study, a follow-up to the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, which compared placebo (PL) versus RLX (60 mg/d or 120 mg/d) on VF risk. BC risk was collected in CORE, and then retrospectively calculated to MORE baseline prior to randomization. The 5-yr BC risk was calculated from the Gail model questionnaire, with scores ≥1.66% considered high risk. Analyses included 3931 women with paired baseline and post-baseline spine radiographs.

Results: The 5-yr predicted baseline BC risks were similar between the PL and pooled RLX groups (mean ± SD; PL, 1.77 ± 0.80; RLX, 1.79 ± 0.89; P=0.44). In the overall analysis cohort, the risk of new VF was decreased by 41% [RR 0.59 (95% CI 0.50, 070)] with RLX, compared to PL. The risks of new VF were similarly decreased by 42% and 32% with RLX, compared to PL, in women with low and high BC risks, respectively (Table). To prevent 1 new VF, 28 and 35 women at low and high BC risk (based on Gail score), respectively, would need to be treated with RLX for 3 yrs.

		Incidence of New Vertebral Fractures at 3 Years				
Breast Cancer Risk Factor		PL (n=1262)	RLX (n=2669)	Relative Risk (95% Confidence Interval)	Absolute Risk	Interaction P-value
Gail score	<1.66% (n=2143)	8.6%	5.0%	0.58 (0.42, 0.81)	3.6%	0.43
	≥1.66% (n=1788)	8.7%	5.9%	0.68 (0.48, 0.96)	2.8%	
Age at randomization	<65 yr (n=1723)	7.9%	4.0%	0.50 (0.34, 0.75)	4.0%	0.26
	≥65 yr (n=2208)	9.2%	6.5%	0.71 (0.53, 0.96)	2.7%	
Number of affected first-degree relatives	0 (n=2985)	11.6%	6.9%	0.60 (0.47, 0.75)	4.7%	0.43
	≥1 (n=492)	10.1%	6.4%	0.63 (0.34, 1.15)	3.8%	

Conclusion: RLX significantly decreased new VF risk in this subset of postmenopausal women, regardless of their BC risk, based on the Gail model. RLX is not approved for BC risk reduction in the U.S.

414

Relationships between Breast Cancer Risk (by Gail Model) and Bone Mineral Density in Postmenopausal Women with Low Bone Mass or Osteoporosis. Mayme Wong, Adeline A Yeo, Yongming Qu, Beth Mitchell, John L Mershon. (SPON: Kelle H Moley). *Eli Lilly and Company, Indianapolis, IN, USA.*

Background: Some studies indicate that women with higher bone mineral density (BMD) have an increased breast cancer (BC) risk than women with low BMD. We examined the relationships between baseline BC risk with either BMD or preexisting vertebral fracture (VF). BC risk was assessed using the Gail model, which estimates the 5-yr predicted invasive BC risk based upon recognized BC risk factors.

Material and Methods: Raloxifene is approved for postmenopausal osteoporosis prevention and treatment. The 3-yr randomized, double-blind Multiple Outcomes of Raloxifene (MORE) study compared raloxifene and placebo on VF risk in women with low BMD or osteoporosis. At MORE baseline, BMD was measured, and spine radiographs were taken to identify preexisting VF. The 5-yr BC risk at MORE baseline was calculated using the Gail model questionnaire, with data collected retrospectively for a subset of women (n=4011).

Results: The 5-yr predicted BC risk in women with and without preexisting VF were similar (1.78% ± 0.79 and 1.79% ± 0.91, respectively, P>0.05). Also, the proportions of women at high BC risk (Gail score ≥1.67%) were 48% and 45% in women with and without preexisting VF, respectively (P>0.05). The proportions of women at high BC risk ranged from 42% to 46%, irrespective of baseline BMD T-score and measurement site. For women without preexisting VF fractures, there was no significant relationship between BC risk and either lumbar spine, or femoral neck BMD, but that between BC risk and total hip BMD was significant (Table).

BMD Measurement Site	Low Bone Mass T-score >-2.5 and ≤-1.0	Osteoporosis T-score ≤-2.5	P-Value
Lumbar Spine	1.80 ± 0.88 (n=970)	1.78 ± 0.97 (n=1384)	0.657
Femoral Neck	1.80 ± 0.96 (n=1780)	1.78 ± 0.82 (n=765)	0.575
Total Hip	1.81 ± 0.92 (n=1903)	1.71 ± 0.79 (n=491)	0.025

Discussion: Postmenopausal women with low bone mass or osteoporosis in MORE have a similar elevated 5-yr BC risk, irrespective of preexisting VF, lumbar spine or femoral neck BMD. Previous analyses showed that baseline femoral neck BMD was correlated with the BC incidence in the MORE placebo group. This data showed no clear relationship between baseline BMD and calculated 5-yr BC risk, suggesting that other factors involved in BC risk are not captured in the Gail model.

415

Atypical Ductal Hyerplasia and High Stromal Angiogenesis in Murine Breast Tissue Lacking Anti-Angiogenic Thrombospondin-1. Beth A Plunkett,[1] Jennifer A Doll,[2] Jack Lawler,[3] Mona Cornwell,[2] Phil Fitchev,[2] Susan E Crawford.[2] (SPON: Serdar E Bulun). *[1]Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [2]Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [3]Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.*

Hypothesis: Breast cancer is dependent on angiogenesis for growth and progression. Thrombospondin-1 (TSP-1), a multifunctional glycoprotein, is a potent inhibitor of angiogenesis that activates TGF-ß *in vitro* and *in vivo.* We postulated that ablation of TSP-1 in the breast promotes epithelial hyperplasia due to loss of inhibitory signals on both endothelial and epithelial cells.

Methods: Breast tissue was harvested from TSP-1 null adult mice (n=4) and compared to age-matched wild type controls (n=2). Angiogenesis was assessed by calculating microvascular density in five non-overlapping high power fields. Endothelial cell positivity was confirmed by using anti-CD31 antibody. Ductal or acinar epithelial changes were evaluated by grading of nuclear atypia and by quantifying immunopositivity for proliferating cell nuclear antigen (PCNA).

Results: The breast tissue of all TSP-1 deficient mice revealed atypical ductal epithelial hyperplasia characterized by increased nuclear-to-cytoplasmic ratios and strong PCNA positivity in glands demonstrating pseudostratification of nuclei and luminal occlusion. All TSP-1 null animals revealed distinctive histologic features in the stromal compartment of the breast. Stromal microvascular density was significantly elevated in TSP-1 deficient mice when compared to controls (17.0 ± 2.9 versus 4.8 ± 0.57, respectively; p=0.004). Vascular ectasia was prominent and more vessels demonstrated smooth muscle cell hyperplasia in the TSP-1 null tissue. Moreover, loss of TSP-1 induced a phenotype in the breast-related adipose tissue consisting of pleomorphic adipocytes and excessive lipid vacuolization.

Conclusions: Thrombospondin-1 is an important negative regulator of the epithelial and stromal compartments of the murine breast. Loss of this potent angiogenic inhibitor triggered atypical epithelial hyperplasia, increased stromal vascularity with ectasia and modulated the adipocyte population. This study suggests that TSP-1 is multifunctional and is a key regulator of stromal-epithelial interactions in breast tissue.

416

Differential Expression of Nestin in the Normal and Preeclamptic Human Placentas. Young-Han Kim,[1] Han-Sung Hwang,[1] Nam-Hoon Cho,[2] Yong-Sun Maeng,[3] Yong-Won Park.[1] (SPON: Brian J Koos). *[1]Obstetrics and Gynecology, Yonsei University Medical College, Seoul, Korea; [2]Pathology, Yonsei University Medical College, Seoul, Korea; [3]Biochemistry, Yonsei University, Seoul, Korea.*

Nestin is a type VI intermediate filament protein originally described in neural stem cells. Recent reports have documented nestin expression in endothelium of newly formed blood vessels, and suggested its role as a marker of capacity for neovascularization and angiogenesis in endothelial cell. The aim of this study was to investigate the differential expression of nestin in the normal and preeclamptic human placentas. Placental tissues from 5 women with severe preeclampsia and 5 gestational age-matched normotensive women were collected at the time of their cesarean section. Western blot analysis for each placental tissue was performed for nestin quantification. Immunohistochemical staining with anti-nestin antibodies was employed to localize nestin positive cells and to investigate differential staining intensity in each placental cell. The nestin protein expression was detected in all of the normal and preeclamptic placental tissues by Western blotting. Compared with the normal placentas, tissues from severe preeclamptic placentas showed higher expression of nestin protein (p<0.001). Nestin immunoreactivity was localized only to endothelial cells of chorionic villi. However, mesenchymal connective tissue cells, cytotrophoblasts, syncytiotrophoblasts and decidual cells did not reveal any specific signal for nestin. We suggest that the capacity for neovascularization and angiogenesis in endothelial cell is increased in preeclamptic placenta compared to that from normal pregnancy. Such changes may be a compensatory mechanism for the reduced materno-fetal exchanges and long lasting fetal hypoxia in preeclamptic pregnancy. Furthermore, these changes in endothelial cell of chorionic villi in preeclamptic pregnancy may give an explanation for fetal response to preeclamptic condition.

417

Rat Placenta Expresses Corticotrophin Releasing Factor Protein and mRNA. Jayaraman Lakshmanan, Edwardo Salido, Fataneh Amidi, Ebraham Amidi, Rama Raj, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: In humans, both the placenta and hypothalamus are sites of corticotrophin releasing factor (CRF) synthesis. Similarly, both placental and hypothalamic CRF have been speculated to function in fetal-maternal stress responses. Previous reports suggested that that the basic machinery governing CRF synthesis is non-existent in rat placenta, and thus this species has not been utilized as a model for the investigation of placental CRF in fetal-maternal stress responses. In the present investigation we sought to demonstrate that rat placenta is the site of CRF synthesis.

Methods: Time-date pregnant Sprague-Dawley rats of e13 were housed individually and provided with food and water ad libitum. On day 16 and 22, maternal rats were anesthetized, abdomen opened, and the fetal placental unit carefully dissected. The placenta was separated and divided into two halves. The first half was fixed in 4% paraformaldehyde and processed for paraffin embedding. The second half was snap frozen in liquid nitrogen and later processed for RNA extraction. Maternal brains were then dissected, cut into two halves and processed as described for the placenta. Tissue sections were subjected to immunohistochemistry with rat CRF antibody (1:250-1:500, Peninsula Laboratory) and the immunoreactive materials on the sections were identified by standard ABC technique. Control sections were incubated without CRF antibody. Purified maternal brain and placental RNA preparations

FRIDAY

were subjected to RT-PCR analysis with primer sequences (Forward: aaagggaaaggcaaagaaa, Reverse: aacacgcggaaaaagttagc) designed with Primer3 software (MIT), using rat CRF cDNA sequences in GeneBank.
Results: Placental sections at e16 and e22 exhibited positive CRF immunostaining in trophoblast cells localized in both the maternal (basal zone) and fetal (labyrinth zone) of the placenta. Control sections showed no immunostaining. RT-PCR analysis identified a single 450bp band both in maternal brain and placental preparations.
Conclusion: Our finding of CRF protein and mRNA expression in rat placenta is consistent with evidence from the human. We speculate that CRF of rat placenta could serve as model for the study of its role in human fetal-maternal stress homeostasis.

418

Prokineticin-1 and Prokineticin Receptor 1 Localisation and Expression in Third Trimester Placenta. Fiona C Denison,[1] Sharon Battersby,[1] Michael Szuber,[1] Margaret J Evans,[2] Henry N Jabbour.[1] (SPON: Hilary OD Critchley). *[1]Centre for Reproductive Biology, University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Pathology, Royal Infirmary, Edinburgh, United Kingdom.*
Objective: To characterise PK1 and PKR1 immunolocalisation, expression and signalling in third trimester human placenta.
Methods: Placentae (n=20) were collected after elective caesarean section at term from women with uncomplicated pregnancies. PK1 and PKR1 were immunolocalised and extracellular regulated signal kinase -1/2 (ERK1/2) phosphorlyation was detected by Western blotting with signalling pathways being dissected using various inhibitors including YM25480, PP2 and AG1478, specific inhibitors of G_q, c-src and epidermal growth factor receptor (EGFR) kinase, respectively. Placental explants (n=6) were treated with 40nM PK-1 and cyclo-oxygenase 2 (COX-2) mRNA expression detected by taqman PCR. PK1 and COX-2 were colocalised using immunofluorescence and confocal microscopy.
Results: PK1 was immunolocalised to endothelium and macrophages in fetal vessels and Hofbauer cells in placental villi. In contrast, PKR1 was predominately localised in syncytial sprouts. ERK-1/2 phosphorylation in placenta was significantly upregulated (5-fold increase; $p<0.05$) following treatment with PK1 for 30 minutes. Dissection of the upstream signalling pathway by the chemical inhibitors demonstrated that PK1 induced phosphorylation of ERK-1/2 was mediated via c-src and EGFR transactivation. Treatment of placenta with PK1 for 4 hours induced a significant increase in COX-2 expression (2.9±0.45 fold increase above control; $p<0.05$). COX-2 expression in response to treatment with PK1 was inhibited following coincubation of the tissue with inhibitors of G_q, c-src, EGFR kinase or ERK1/2. Using double immunofluorescence, co-localisation/co-expression of PK-1 and COX-2 was demonstrated in various cellular compartments within the placenta including trophoblast and macrophages.
Conclusions: The cellular immunolocalisation of PK1 and PKR1 within placenta and upregulation of COX-2 by PK1 is supportive of PK1 being involved in placental vascular physiology. In addition, expression of PKR1 in syncytial sprouts, which characterise areas of hypoxia and immature villous formation, suggest that another role of PK1 may be in mediating trophoblast differentiation in response to hypoxia.

419

Expression of S1P Synthetic Enzymes and Cognate S1P Receptors at the Human Maternal: Fetal Interface. Malgorzata E Skaznik-Wikiel,[1] Ling Zhang,[1] Yoshiaki Kanda,[1] Drucilla J Roberts,[2] Jeffrey L Ecker,[*1] James K Pru.[1] *[1]Vincent Obstetrics and Gynecology Service, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.*
Objective: Previous studies in mice (Skaznik-Wikiel, *Biol Reprod*; 74:569) showed induction of the sphingolipid signaling pathway during early pregnancy at the maternal:embryonic interface. The current studies were completed to examine this sphingolipid pathway, involving enzymes that generate sphingosine-1-phosphate (S1P), as well as cognate S1P receptors, in tissues from human pregnancy.
Methods: Immunohistochemistry, RT-PCR and western analysis were used to study expression of sphingolipid metabolizing enzyme and S1P receptors in the human placenta, choriocarcinoma cell lines, and maternal decidual tissue.
Results: In placenta sphingosine kinase (SphK1) protein, an enzyme that converts sphingosine to S1P, was abundantly expressed in the muscular compartment of placental arteries. The S1P receptor $S1P_2$ was found to be expressed on both sides of the maternal:fetal interface. Decidualized uterine stromal cells robustly expressed $S1P_2$, and this receptor localized to stromal and syncytiotrophoblast cells of chorionic villi. S1P receptors $S1P_1$, $S1P_2$, and $S1P_3$ were found by RT-PCR analysis to be expressed in Jeg-3 and JAR choriocarcinoma cell lines. Immunocytochemical staining revealed that $S1P_2$ protein was robustly expressed in Jeg-3 cells. Treatment of Jeg-3 cells with S1P resulted in activation of the ERK signaling pathway demonstrating that S1P receptors were not only present on these trophoblast cell lines, but that they were also functional.
Conclusions: Our studies localize enzymes that generate S1P and S1P receptors at the human maternal:fetal interface. Within the placental vasculature, we postulate that through local S1P production, SphK1 activity is an important determinant in regulating vasculare tone within the placenta. Vascular-derived S1P may also regulate trophoblast differentiation in chorionic villi, a possibility supported by findings of $S1P_2$ protein on both placental syncytiotrophoblast and Jeg-3 cells, as well as S1P-induced activation of ERK signaling in Jeg-3 cells. Our findings highlight the importance of continued studies in primates and humans since differences exist in expression of sphingolipid related proteins between mice and humans. Supported by Vincent Memorial Research Funds.

420

Regulation of Angiopoietin I and II Gene Expression by CGRP and ADM at the Human Implantation Site. Hong Y Wen, Xin Ma, Manubai Nagamani,[*] Chandrasekhar Yallampalli,[*] Yuan L Dong.[*] *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
OBJECTIVES: Angiopoietin I (Ang-I) and angiopoietin II (Ang-II) are two important angiostimulatory factors expressed by placental trophoblast cells. Both of them bind to the Tie-2 tyrosine kinase receptor on vascular endothelial cells, work in concert with vascular endothelial growth factor (VEGF) to regulate vascular morphogenesis, remodeling and maturation during pregnancy. However, their regulation at the human implantation site is yet to be determined. Present study was designed to determine whether adrenomedullin (ADM) and calcitonin gene-related peptide (CGRP), which were expressed by trophoblast inhuman placenta, regulate Ang-I and -II gene expression at the implantation site during early pregnancy.
METHODS: First-trimester villous tissues were obtained from women undergoing elective legal pregnancy termination. The villous tissues or extravillous trophoblast cells (HTR-8/SV neo cell line) were cultured in RPMI 1640 in standard tissue culture condition in the presence or absence of ADM or CGRP. The mRNA expression of Ang-I and Ang-II were determined by RT-PCR. Meanwhile, the angiostatic factor soluble fms-like tyrosine kinase 1 (sFlt-1) secreted by trophoblasts in culture media was determined using enzyme immunoassay (ELISA).
RESULTS: 1) mRNA expression for Ang-I by HTR-8/SV cells is significantly stimulated by CGRP (10^{-8} M) treatment ($p<0.05$) at 48h of culture and this stimulation is completely blocked by CGRP antagonist $CGRP_{8-37}$ (10^{-7} M) ($p<0.01$); 2) Ang-II mRNA expression is stimulated by CGRP treatment but this increase does not reach the significant level ($p>0.05$); 3) Treatment of the cells with ADM failed to alter Ang-I and -II mRNA gene expression ($p>0.05$); 4) In human first-trimester villous tissue culture, Ang-I mRNA was increased by CGRP (10^{-8} M) ($p<0.05$) and Ang-II mRNA levels were unaltered; and 5) ADM stimulates Ang-I mRNA expression by villous explant culture but inhibits Ang-II expression in a dose-dependent manner ($p<0.05$, ANOVA).
CONCLUSION: Both CGRP and ADM regulate mRNA levels for Ang-I and Ang-II, favoring placental angiogenesis and vascularization. Thus, locally produced CGRP and ADM at the human implantation site may play a role in placental development and fetal growth during early pregnancy.

421

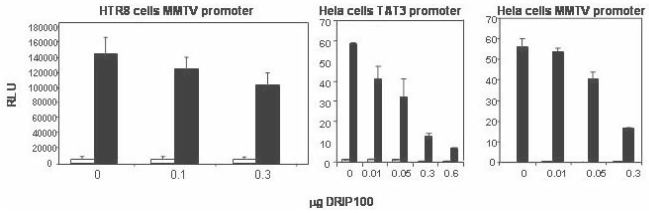
DRIP100 Is a Cofactor for the Glucocorticoid Receptor in the Human Placenta. Weiwei Chen,[2] Naima Ismaili,[2] Caroline Tang,[1] Men-Jean Lee.[*1] *[1]Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Microbiology, New York University, New York, NY, USA.*
Objective: The glucocorticoid receptor (GR) controls the expression of specific sets of genes in distinct cell types. GR promoter-specific gene regulation is generated through interactions with specific combinations of regulatory proteins termed transcriptional cofactors. Changes in levels of specific cofactors have

been linked to complications during pregnancy, including placental pathology, such as IUGR. Our objective is to identify GR cofactors from the placenta that may impact GR activity and placenta function.

Methods: To identify cofactors that regulate GR activity in the placenta, we carried out a yeast two-hybrid screen from a human placenta cDNA library using the GR-ligand-binding domain as bait. Seventeen positive clones were identified. The corresponding DNA fragments were sequenced, and products identified by a search through the NCBI database. We validated putative cofactor interaction with GR using both *in vitro* GST pull-down and *in vivo* co-immunoprecipitation assays. The effect of the cofactor on GR transcriptional activity was determined by co-transfecting either HeLa, U2OS-hGR, or HTR8 cells (a first trimester extravillous trophoblast cell line) with an expression vector for the putative cofactor in the presence of 2 different GR-responsive luciferase reporter genes.

Results: Sequence analysis revealed that one of the cDNA fragments corresponds to the transcriptional cofactor DRIP100 (MED24). DRIP100 repressed GR transactivation in a dose dependent manner in both U2OS-hGR (not shown), HeLa, and HTR8 cells (Figure 1). We further demonstrated that the carboxyl-terminal region of DRIP100 is sufficient for interaction with GR.

Conclusion: GR binds to DRIP100 and overexpression of DRIP100 reduces GR transcriptional activity in a variety of cell types including a human trophoblast cell line. Future studies are directed toward understanding the mechanisms by which DRIP100 limits GR activity as a corepressor and could affect placental development.



422

Adrenomedullin Enhances Angiogenic Growth Factor Expression the Human First-Trimester Villous Explants. Hong Y Wen, Xin Ma, Manubai Nagamani,* Hui-Qun Wang, Chandrasekhar Yallampalli,* Yuan L Dong.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

OBJECTIVES: Normal placentation requires sufficient cytotrophoblast invasion into the uterine wall and subsequent remodeling of maternal uterine vasculature. In this process, trophoblast cell-derived angiogenic growth factors, vascular endothelium growth factor (VEGF), plays a key role in promoting placental vascularization. However, the regulation of VEGF at the implantation site remains unclear. The present study was designed to determine the co-localization of VEGF, adrenomedullin (ADM) and its receptors at the human feto-maternal interface and examine the effect of ADM on angiogenic growth factor expression during early pregnancy.

METHODS: Villous tissues were obtained from women undergoing first trimester legal pregnancy termination. The cellular localization of VEGF, PlGF, ADM and its receptor components calcitonin receptor-like receptor (CRLR) and receptor activity modifying protein 2 and 3 ($RAMP_2$ & $_3$) was determined by immunohistochemistry. The villous tissues and extravillous trophoblast cells (HTR-8/SV neo cell) were cultured in RPMI 1640 in the presence or absence of ADM. The expression of mRNA for VEGF and angiostatic factors soluble fms-like tyrosine kinase (sFlt-1) were determined by RT-PCR. The concentration of sFlt-1 in villous explant culture media was determined using enzyme immunoassay (ELISA).

RESULTS: 1) Immunohistochemical studies showed that VEGF is co-localized with ADM, and its receptor components CRLR/$RAMP_2$ and $RAMP_3$ on both villous and extravillous trophoblast cells in first trimester villous tissues; 2) VEGF mRNA expression by HTR-8/SV neo cell line was significantly increased by ADM in a dose dependent pattern (24 h; $p<0.05$); 3) sFlt-1 mRNA expression by HTR-8/SV neo cells was significantly suppressed by ADM (10^{-8} M) after 24h treatment ($p<0.05$), while without change in the full length Flt-1 ($p > 0.05$); and 4) In human first-trimester villous tissue ADM (10^{-8} M) failed to significantly stimulate mRNA expression for VEGF ($p > 0.05$), however, ADM (10^{-8} M) significantly inhibited both sFlt- mRNA and protein secretion in the culture media ($p < 0.01$).

CONCLUSION: This study supports the hypothesis that ADM may stimulate trophoblast cell-derived angiogenic growth factors and inhibit angiostatic growth factor during early pregnancy, thus playing a role in placental vascular development and fetal growth.

423

Hypoxia Enhances the Expression of Follistatin-Related Gene (FLRG) in Cultured Term Human Trophoblasts. Tal Biron-Shental, Eli Rimon, Anthony L Shanks, Tammy Shim, D Michael Nelson,* Yoel Sadovsky.* *Obstetrics & Gynecology and Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO, USA.*

Objective: Exposure of human trophoblasts to hypoxic injury diminishes trophoblast differentiation. FLRG belongs to the TGFβ family of proteins It is expressed in the placenta, and regulates growth and differentiation of other tissues, likely by blocking activin activity. Based on a microarray screen of human trophoblasts we hypothesized that hypoxia enhances the expression of FLRG in primary term human trophoblasts.

Methods: Cytotrophoblasts were isolated from normal term placentas using enzymatic digestion and a Percoll gradient. Trophoblasts were cultured for 48 h in either standard conditions (FiO2=20% oxygen) or hypoxia (FiO2<1%). RT-qPCR and western immunoblotting were used to measure cellular FLRG mRNA and protein, respectively.

Results: We found that the basal expression of FLRG in cultured primary human trophoblasts was stable over the culture period. Hypoxia upregulated FLRG mRNA in a time-dependent manner, with a 2-4 fold increase within 4 h of hypoxia, and a maximal increase (>6-fold) within 24 h in culture. Interestingly, cellular FLRG protein increased after 4 h but decreased within 48 h in hypoxic conditions. The exposure of plated trophoblasts to either the hypoxia-mimetic cobalt chloride (0.1-0.4 mM) or the proline hydroxylase inhibitor dimethyloxaloylglycine (DMOG, 0.125-1 mM), both known to stimulate HIF-1α activity, upregulated FLRG expression in a concentration dependent manner.

Conclusions: Hypoxia enhances the expression of FLRG mRNA and protein in cultured primary human trophoblasts. Our finding that hypoxia-mimetics enhance FLRG expression implicates HIF-1α in this process.

424

Regulation of COX-2 and PGDH mRNA Expression in Human Chorion Trophoblast Cells: Role of Calcium, PKC and MAPKs. Valentina Casciani,[1,2] Emanuela Marinoni,*[1] Romolo Di Lorio,*[1] Massimo Moscarini,[1] John RG Challis.*[2] *[1]Department of Physiology, University of Toronto, Toronto, ON, Canada; [2]Gynecology, Perinatology and Child Health University, University of Rome "La Sapienza", Rome, Italy.*

Introduction: During human labor, Prostaglandins (PGs) act as stimulators of myometrial contractility, fetal membranes rupture and cervical ripening. The amount of PGs is determined by the expression and activity of COX-2 and PGDH, which catalyze respectively the limiting reactions of PG synthesis and metabolism. COX-2 and PGDH are both expressed in human chorion. We previously observed in chorion trophoblasts that calcium ionophore A23187 up-regulates COX-2 and down-regulates PGDH protein and mRNA expression. We investigated on the role of PKC, target of calcium, and of MAPKs (p38, JNK and ERK1/2) in COX-2 and PGDH gene expression regulation.

Methods: Primary cultures of human chorion trophoblasts were treated as follow: 1) PKC activator phorbol ester PMA 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M for 24h; 2) PMA 10^{-7}M for 1h, 2h, 4h, 8h and 24h; 3) PMA (10^{-7}M) in presence of the PKC inhibitor GF109203X (10^{-5}M) for 8h; 4) A23187 (10^{-6}M) or PMA (10^{-7}M) in presence of p38, JNK or MEK1/2 inhibitors (SB203580, SP600125 and U0126, 10^{-5}M) for 8h. COX-2 and PGDH expression in response to treatments was assessed with Real Time PCR. GAPDH and β-actin were used for data normalization.

Results: PMA up-regulated COX-2 and down-regulated PGDH mRNA expression in a dose- and time-dependent manner. GF109203X reversed PMA effect on both enzymes. SB203580 attenuated the effect of PMA and A23187 on COX-2. U0126 attenuated the effect of PMA, but not of A23187, on COX-2. SP600125 apparently had no effect on COX-2 expression. All MAPKs inhibitors failed to reverse the negative effect of either A23187 or PMA on PGDH.

Discussion: Calcium influx and PKC activation induce COX-2 and simultaneously inhibit PGDH mRNA expression. p38 MAPK attenuated the positive effect of A23187 and PMA on COX-2 expression. We speculate that an increase in intracellular calcium activates calcium-dependent PKC isoforms which, in turn, acting via p38 MAPK, induce COX-2 mRNA expression. MEK1/2 inhibitor partly inverted the effect of PMA on COX-2, but not the effect of the ionophore. Therefore, we cannot exclude the involvement of calcium-independent PKC isoforms able to induce COX-2 via MEK1/2 activation. The intracellular pathway controlling PGDH inhibition in response to calcium influx and PKC activation does not involve MAPKs.

FRIDAY

425

Mechanisms Regulating Glucocorticoid Receptor (GR) Function under Hypoxic Conditions: Implications in Intrauterine Growth Restriction (IUGR). Men-Jean Lee,* Caroline Tang, Yuehong Ma, Seth Guller.* *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

OBJECTIVE: We have previously reported that glucocorticoid (GC)-dependent downregulation of GR expression (i.e. homologous down-regulation) in placental fibroblasts was delayed under hypoxic conditions (JSGI 2005, Abstract#672). Since excessive periplacental levels of GC accompany hypoxic conditions in IUGR, we sought to explore molecular mechanisms through which hypoxia enhances GR function.

METHODS: HTR-8/SVneo (HTR8) cells (1st trimester extravillous trophoblast cell line), placental fibroblasts, and U2OS cells that have been stably-transfected to express hGR (U2OS-hGR) were treated with 100nM DEX versus ETOH control under normoxic (20% O2) and hypoxic (1-2% O2) conditions from 2-48 hours. Western blot analysis was performed to examine levels of total GR protein expression. U2OS-hGR cells were cultured on glass chamber slides until 60% confluent and treated as above to determine the cellular localization of GR and phosphorylated GR (i.e. GR-phospho226, a phosphorylated form of GR that is translocated to the nucleus upon DEX treatment). Fluorescence immunocytochemistry was performed using an antibody for total GR and a GR-phospho226 antibody. A luciferase assay was performed under similar conditions using the U2OS-hGR cells with a GR-responsive MMTV-luciferase reporter gene.

RESULTS: Hypoxic treatment delayed the DEX-mediated downregulation of GR protein expression at 24-48 h by 2-4 fold in cultures of placental fibroblasts, HTR8 cells, and the U2OS-hGR cell line. Conversely, treatment with DEX for 24-48h enhanced GR-mediated transcriptional activation, and hypoxia did not affect this response. Total GR and GR-phospho226 were translocated to the nucleus following a 2-4 h DEX treatment, and this effect was no longer seen after a 24 h DEX exposure. However, under hypoxia, the nuclear localization of total GR and GR-phospho226 was maintained after 24 h of DEX treatment.

CONCLUSIONS: Our results indicate that hypoxic conditions may enhance GC action at the uterine-placental interface by prolonging the nuclear localization and/or stability of phosphorylated GR. This suggests a mechansim through which GC action is aberrantly enhanced in placentas in pregnancies with IUGR.

426

Interaction between Lipoxygenase Pathway and Progesterone on 11β-Hydroxysteroid Dehydrogenase Type 2 in Cultured Human Term Placental Trophoblast. Kazuyo Sato,[1,2] Kunihiro Okamura,*[2] John RG Challis.*[1] *[1]Dept. of Physiology, Ob/Gyn and Medicine, Univ. of Toronto, Toronto, ON, Canada; [2]Dept. of Ob/Gyn, Tohoku Univ. Grad. Sch. Med, Sendai, Miyagi, Japan.*

Objective: Glucocorticoids (GCs) play an important role in not only parturition but also fetal growth and maturation. However, excessive amounts of GCs may lead to preterm labor and intrauterine growth restriction. 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) exhibits only oxidase activity (cortisol to cortisone, its biologically inactive metabolite) under physiological conditions, and acts as a placental barrier to protect the fetus from high levels of maternal GCs. On the other hand, progesterone (P4) is one of major steroid products of the human placenta that play an integrative role in the regulation of pregnancy maintenance and fetal maturation, and has been reported as a potent inhibitor of 11β-HSD2.

In the event of intrauterine infection, eicosanoids including lipoxygenase (LOX) metabolites are produced in placenta, and these may contribute to preterm labor and adverse fetal outcomes. We reported previously (at SGI 2006) that LOX pathway regulated both 11β-HSD2 activity and endogeneous P4 production. However, it is still unclear that the effect of LOX pathway on 11β-HSD2 might be mediated through P4. Therefore, we have evaluated the interaction between LOX pathway and P4 on 11β-HSD2 in cultured human placental trophoblast.

Methods: Placental trophoblast cells were isolated from human term placenta, and were treated with leukotriene B_4 (LTB_4), 12(S)-hydroxyeicosatetraenoate (12-HETE), nordihydroguaiaretic acid (NDGA), P4 and RU486. 11β-HSD2 activity was determined by radiometric conversion assay. 11β-HSD2 expression was evaluated by Western blot analysis.

Results: 11β-HSD2 activity was significantly down-regulated with P4 and up-regulated with NDGA or RU486. It was also up-regulated with NDGA plus RU486, but the effect was not significantly enhanced by RU486. 11β-HSD2 protein expression was also decreased with P4, and increased with NDGA or RU486. LOX metabolites reduced both the level of 11β-HSD2 activity and protein expression with NDGA or RU486, but there was no significant change with LOX metabolites alone.

Conclusion: Both LOX pathway and P4 is involved in the regulation of 11β-HSD2 in human placental trophoblast cells. However, the effect of LOX pathway mediated through P4 might be much smaller than the direct effect of LOX pathway.

427

Adrenomedullin Receptor in Cultured Trophoblast and Amnion Cells: Effects of Steroid Hormones. Emanuela Marinoni,* Katia Pacioni, Alessandra Sambuchini, Francesca Ciardo, Giovanna Corona, Alessandra Petrilli, Valentina Loguercio, Romolo Di Lorio.* *Dept. of Gynecology, Perinatology and Child Health, University of Rome "La Sapienza", Rome, Italy.*

Objective: In human pregnancy adrenomedullin (AM) is produced by the placenta and fetal membranes and is implicated in different functions from implantation to delivery. We have recently demonstrated that AM receptor (AM-R) is localized in intrauterine tissues and that its distribution changes with gestational age. There is evidence that both the peptide and its receptors are regulated by endocrine factors. AM synthesis by cytotrophoblast cells in culture is stimulated by glucocorticoids. In the rat uterus AM-R levels are regulated by steroid hormones. We speculate that in placental tissues steroid hormones may modulate AM-R expression, regulating, in turn, AM activity. In the present study we investigated the effects of betamethasone and progesterone on AM-R in trophoblast and amnion cells.

Methods: Primary cultures of cytotrophoblasts were isolated from human term placentas (n=6) and were maintained in serum-free medium for up to 96 h with and without 10^{-7} M betamethasone (BETA), and progesterone (P4). Corion trophoblast and amnion cells were obtained by term fetal membranes (N=6). Time response to BETA, and P4 was performed with the concentration of 10^{-7} M at 2h, 8h and 24h. The effect of treatment at each time point was compared to control sample. Immunocytochemical analysis was performed by the avidin/biotin immunoperoxidase method using a specific antibody to AM receptor (dilution 1:400).

Results: AM-R was localized on either placenta and chorion trophoblast cells and in amnion cells of fetal membranes. The prevalence of immunoreactive positive cells for AM-R and the intensity of the staining was not affected by BETA or P4 treatment at any time-point.

Conclusion: The presence of binding sites in the placenta and fetal membranes indicates that AM-R might modulate AM function within fetoplacental tissues. However, differently from AM, AM-R expression in human placenta and fetal membranes seems not to be regulated by steroid hormones. This finding may suggest that steroid hormones affect AM function in pregnancy acting on the synthesis and release instead of on AM-R binding.

428

Localization of Key Growth Signaling Proteins in Placentomes of Overfed Ewes. Mei J Zhu,[1,2] Min Du,[1,2] Bret W Hess,[1,2] Peter W Nathanielsz,*[1,3] Stephen P Ford.*[1,2] *[1]Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA; [2]Department of Animal Science, University of Wyoming, Laramie, WY, USA; [3]Center for Pregnancy and Newborn Research, Department of Obstetrics & Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: In ruminants, placentomes, composed of a fetal portion (cotyledon, COT) and maternal portion (caruncle, CAR), promote fetal nutrient uptake. Accelerated fetal growth is associated with up-regulation of placental nutrient transport. Mammalian target of rapamycin (mTOR) pathway is an important nutrient sensing pathway in mammalian cells and MAPK/ERK1/2 and PI3K/Akt growth signaling pathways are linked to placentomal vascularity. Thus these pathways may play a role in placentomal nutrient transfer. **Objective:** Localization and quantitation of mTOR, ERK1/2 and Akt in the placentomes from overfed vs. control fed ewes. **Methods:** Ewes were randomly assigned to a control (C, 100% of NRC recommendations, n=5) or obesogenic (OB, 150% NRC, n=5) diet from 60 days before to 75 days after conception when they were euthanized. COT and CAR tissues were frozen in liquid nitrogen for later protein extraction and Western analysis, and placentomes were fixed with paraformaldehyde and paraffin embedded. Paraffin sections were used to localize mTOR, Akt and ERK and their phosphorylated forms in COT and CAR tissues using specific antisera and for vascular density via image analysis. **Results:** OB and C ewes increased their body weight by ~50% and ~7% from diet initiation to necropsy. Fetuses from OB ewes were ~30%

heavier (P<0.05) than those from C ewes on day 75 (374 ± 10 vs. 268 ± 12 g). Preliminary data suggest a reduction (P<0.10) in COT mTOR, Akt and ERK and their phosphorylated forms, in association with reduced vascularity in placentomes of OB vs. C ewes. Immunohistochemical staining revealed that mTOR, Akt, ERK1/2 and their phosphorylated forms were localized in the endothelium and smooth muscle cells of COT and CAR blood vessels. In the placentomal tissues other than vessels, total mTOR, total Akt and total ERK and their phosporylated forms were localized in both cytoplasm and nucleus of trophoblast cells at the fetal:maternal interface. **Conclusion:** mTOR, Akt, ERK1/2 and their phosporylated forms were mainly located in placentomal blood vessels and trophectoderm where they could play an active role in nutrient sensing and transport. NIH INBRE 1P20RR16474.

429

Global Analysis of Genomic Methylation in First Trimester Trophoblast. Lucia Brown,[1] Odelia Nahum,[2] Gregory Brown,[3] Stephen Brown.[1] (SPON: Mark Phillippe). *[1]Ob/Gyn, University of Vermont, Burlington, VT, USA; [2]Pathology, Columbia University, New York, NY, USA; [3]Pathology, Columbus Regional Hospital, Columbus, IN, USA.*

Hypothesis: We hypothesize that DNA methylation plays a significant role in trophoblast biology.

Objective: To perform a genome-wide assessment of genomic DNA methylation in human trophoblast.

Although its precise biologic role is not well known, genomic methylation is thought to play an important role in the regulation of gene expression and in chromatin structure in mammals. Anecdotal comments as well as studies from the early 1980s suggest that human trophoblast DNA is substantially less methylated than DNA from other sources, but these data have never been confirmed, and a systematic determination of the extent and distribution of methylation in human or other mammalian trophoblast has never been performed.

Methods and Results: We have developed a novel method, based on methylation-specific amplification followed by comparative hybridization, that allows genome-wide comparisons of methylation between DNA samples from different tissues. We have used this method to perform a global comparison of CpG methylation between first trimester trophoblast and DNA derived from lymphocytes and other sources. Our results indicate that, overall, trophoblast DNA is indeed hypomethylated. Surprisingly, hypomethylated segments are largely concentrated in specific chromosomal regions such that each chromosome has its own specific pattern. Some chromosomes (eg 16 and 21) show much higher levels of hypomethylation than others. Oligonucleotide microarray analysis of chromosome 21 indicates that approximately 1000 segments out of a total of 46,470 tested (~2%) show strong evidence of hypomethylation and that these are clustered in two regions near the centromere and telomere. Hypomethylated segments have a ~5 fold higher than expected probability of occurring within expressed sequence (known genes and/or ESTs), further corroborating the highly non-random nature of trophoblast hypomethylation. Unexpectedly, small regions of relative hyper-methylation are also present as well.

Conclusions: Our data strongly support the idea that DNA methylation plays a significant role in the trophoblast. As a first step towards understanding that role, we plan future studies aimed at understanding how methylation changes as a function of gestational age as well as disease such as pre-eclampsia and IUGR.

430

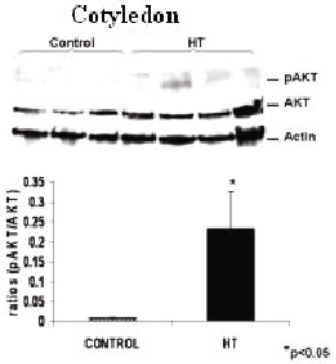
Placental Expression of Phosphorylated ERK and AKT Protein Near-Term in an Ovine Model of Intrauterine Growth Restricition (IUGR). Juan A Arroyo,[1] Russell V Anthony,[2] Thomas Parker,[2] Henry L Galan.*[1] *[1]Obstetrics and Gynecology, University of Colorado and Health Sciences Center, Denver, CO, USA; [2]Pediatrics, University of Colorado and Health Sciences Center, Denver, CO, USA.*

Introduction: eNOS is an enzyme known to regulate blood flow that is modulated by shear stress. Shear stress also modulates the level of activation (phosphorylation) of ERK1/2 and AKT in different tissues. In an established hyperthermic (HT) ovine model of fetal growth restriction (FGR), we hypothesize that phosphorylated (p)ERK1/2 and/or AKT protein levels would be increased in the placenta and that this correlates with an increase in placental eNOS protein observed in these tissues at 130 days of gestation (dGA) (near-term) animals.

Study designs: 4 ewes were exposed to HT conditions for 80 days to induce FGR and 4 were used as controls in ambient conditions. Umbilical artery Doppler measurements were performed and aortic catheters were placed for blood gas analysis and systemic pressure determination. Placentomes were separated into caruncle and cotyledon components and used in Western blot analysis with antibodies against eNOS, pERK, total ERK, pAKT and total AKT.

Results: Compared to controls, HT pregnancies showed: 1) smaller fetuses (p≤0.03) and placentae (p≤0.03), 2) significantly higher S/D ratios (p<0.009) and higher systemic blood pressures (p<0.03), 3) decreased fetal O_2 saturation (p<0.008) and O_2 levels (p<0.002), 4) a 1.5-fold increase in eNOS protein concentration (p<0.04) in the cotyledon tissues with no significant differences for the caruncle tissues, 5) a 1.5-fold trend of increase in pERK (p<0.08) in the caruncle tissues with no differences in pAKT, 6) a significant increase of pAKT protein (p<0.05) in the cotyledons but no differences of pERK protein expression in this tissue.

Conclusion: pERK protein expression was increased near term in the caruncle (maternal compartment) and pAKT protein was increased in the cotyledon (fetal compartment) of HT animals and this increase may be secondary to shear stress at term as suggested by abnormal Doppler and systemic hypertension (Supported by NIH grant R01 HL071990-01A1).



431

The Role of the Tyrosine Phosphatases SHP-1 and SHP-2 in Regulating Placental Cytotrophoblast Proliferation. Gemma West, Karen Forbes, Melissa Westwood, John D Aplin. (SPON: Philip Newton Baker). *Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom.*

Background: In the human placenta, syncytiotrophoblast has a key role in transporting solutes, hormone production and as the primary maternal-fetal barrier. Syncytiotrophoblast is formed by the proliferation, differentiation and fusion of the underlying villous cytotrophoblast (CTB) and although a balance between these processes is critical for normal placental development, their regulation is poorly understood. In many cellular systems, IGF1R-activated signalling events are important in proliferation and recently the tyrosine phosphatase (PTP) SHP-2 has been implicated in these pathways. Although SHP-2 is highly transcribed in placenta, its cell localisation and role are unknown. mRNA encoding a structurally similar PTP, SHP-1, is also present; in other systems SHP-1 inhibits cell proliferation, but again, its function in placenta is unclear.

Objective: To examine the expression and function of SHP-1 and SHP-2 in the human placenta.

Methods: We have developed an explant model of human placenta in which the spatial and ontological relationships between cells in the villus are maintained, in order to examine the regulation of placental cell function. Immunohistochemistry (IHC) and Western blotting of placenta explants was undertaken to examine expression and localisation of SHP-1 and SHP-2 in the placenta. To investigate the role of these PTPs in regulating CTB proliferation, explants were cultured in serum free DMEM/F12, containing BrdU, +/- 10nM IGF-I for up to 4 days. Levels of proliferation in the tissue was then analysed by IHC using specific antisera for the proliferative markers, BrdU and Ki67.

Results: We have established that both SHP-1 and SHP-2 proteins are expressed in the human placenta. SHP-1 is primarily localised to the CTB whereas SHP-2 is present in CTB and, albeit more weakly, in syncytiotrophoblast. Proliferation assays revealed that CTB proliferation increased during 4 days in culture and was further increased by IGF-I. As proliferation increased, CTB expression of SHP-1 decreased whereas SHP-2 was upregulated.

FRIDAY

Conclusions: Our data demonstrate for the first time, the localisation and modulation of SHP-1 and SHP-2 in the human placenta. We suggest opposing roles for these PTPs in regulating CTB proliferation. Function blocking experiments are planned to confirm that SHP-2 is a positive regulator, and that SHP-1 is involved in negatively regulating proliferation.

432

Urocortin 2 and Urocortin 3 Expression Is Regulated by HIF-1a in First Trimester Placenta Explants. Alberto Imperatore,[1,2] Felice Petraglia,[*1] John RG Challis,[*2] Isabella Caniggia.[*3] *[1]Dept of Physiology, Obgyn and Medicine, University of Toronto, Toronto, Canada; [2]Department of Pediatrics, Obstetrics, and Reproductive Medicine, Policlinico Le Scotte, University of Siena, Siena, Italy; [3]Dept of Obgyn, Samuel Lunenfeld Research Institute, Toronto, Canada.*

Objective: urocortin 2 (Ucn2) and urocortin 3 (Ucn3) are new members of the CRH family of peptides binding selectively the CRF-R2. Although their expression and localization in the human placenta has been described their regulation remains unknown. The aims herein were to investigate whether Ucn2 and Ucn3 expression *in vitro* were influenced by low-oxygenation condition and to understand the role of HIF-1a in regulating their expression. **Methods:** cell culture and first trimester explants were obtained from elective termination of pregnancy and incubated overnight at 37°C in standard condition (20%O_2) to allow attachment then placed in an atmosphere of 8% or 3%O_2 (48h at 37°C). Moreover, placental explants were exposed to dimethyloxalyl-glycin(DMOG), an inhibitor of prolyl-hydroxylases, which mimics hypoxia by increasing HIF-1a stability and to determine if intermittent change in oxygenation affects Ucn2 and Ucn3 expression hypoxia/re-oxygenation(HR) was performed. Total mRNA was collected for real-time qPCR evaluation. **Results:** increased Ucn2-Ucn3 expression occurs when oxygen tension is lower. Ucn2-Ucn3 expression levels from the cell culture where ten to thirtyfold higher when exposed to 3%O_2 compared to standard conditions. Furthermore, significantly higher mRNA expression was found when first trimester explants were kept in hypoxic environment. Moreover, Ucn2 and Ucn3 expression by DMOG-treated explants was significantly greater than that of explants kept at 20% and that Ucn2-Ucn3 transcript levels in first trimester explants exposed to hypoxia-reoxygenation (HR) were higher than in control. **Discussion:** Ucn2-3 expression is sensitive to O_2 tensions and regulated by HIF-1a. Results in first trimester placental explants, a unique model to study O_2 influence as driving force in human trophoblast cell differentiation, development and function allows us to speculate that these peptides might act as differentiating and proliferating factors in low oxygen conditions while their increase in hypoxia-reoxygenation may indicate their involvement in pathological oxidative stress-related conditions as preeclampsia.

433

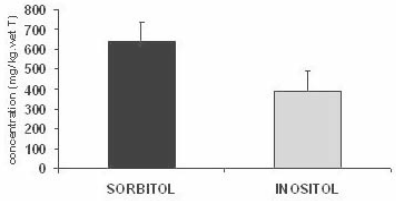
Determination of the NAFT5/TonEBP Transcription Factor in the Human Placenta. Juan A Arroyo,[1] Bradley Ziebell,[1] Cecila Teng,[2] Henry L Galan,[*1] Frederick C Battaglia.[*2] *[1]Obstetrics and Gynecology, University of Colorado and Health Sciences Center, Denver, CO, USA; [2]Pediatrics, University of Colorado and Health Sciences Center, Denver, CO, USA.*

Introduction: In many tissues, osmotic stress causes the accumulation of organic osmolytes such as inositol and sorbitol. Previous studies in our laboratory demonstrated a significant umbilical uptake of sorbitol in both the ovine and human fetal circulations. In addition, we have found high free sorbitol concentrations within the ovine placenta. The induction of the genes for sorbitol synthesis from glucose occurs at the transcriptional level and is mediated by the transcription factor NFAT5/TonEBP. In this study, we tested for the presence of the NFAT5 mRNA and protein levels in the human placenta and will confirm the levels of sorbitol and inositol in the ovine placenta.

Study design: Normal human placentae and ovine placentomes were collected and snap frozen in liquid nitrogen. RNA was isolated and cDNA made. RT-PCR was done using primers from the human NFTA5 sequence. Western blot was performed for protein analysis with an antibody against human NFAT5. Actin westerns on the same blots were used to account for inter-lane loading variation. HPLC was used to determine the levels of inositol and sorbitol in the ovine placentae.

Results: We demonstrated: 1) NFAT5 mRNA in the human and ovine placenta 2) NFAT5 protein in the human placenta, but not in the ovine placenta, 3) significant high levels of sorbitol (641.0 mg/kg wet T) and inositol (388.9mg/kg wet T) in the ovine placenta.

Conclusion: The *in* vivo studies of sorbitol and inositol umbilical uptake led us to determine the presence of NFAT5 at the level of mRNA and protein in the human placenta and mRNA in the sheep placenta. The finding of this transcription factor has not previously been reported in the placenta. We also observed high levels of sorbitol and inositol in the ovine placenta suggesting the presence of a mechanism of induction for these molecules. Presence of NFAT5 in the placenta suggests an involvement for this protein in the induction of these osmolites (Supported by NIH grant R01 HDO34837-08).



434

Gene Profiling of Human Decidual Basalis and Parietalis. Joseph Huang, Irina A Buhimschi, Catalin S Buhimschi, Edward Kuczynski, Seth Guller,[*] Charles J Lockwood.[*] *Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA.*

Objective: Decidua is the maternal portion of gestational tissue derived from endometrium that plays a crucial role in communication between mother and fetus. At term, two distinct areas of decidua are identified: decidual basalis (DB) of basal plate and decidual parietalis (DP) of fetal membranes. We hypothesized that differential decidual localization is accompanied by distinct profiles in gene expression.

Methods: Microarray analysis was employed to demonstrate gene expression profiles for DB, DP and placental villous tissue (VT) carefully dissected from placentas and fetal membranes of patients at term without labor (n=4). Total RNA from each specimen was hybridized to an Affymetrix HG_U133 Plus 2.0 chip containing approximately 47,400 human genes and expressed sequence tags (ESTs). The microarray results were analyzed with GCOS 1.4 and GeneSpring GX 7.3.1 software. After per chip and per gene normalization as well as exclusion of genes absent in all samples, differences and correlations among different tissues were analyzed by one-way ANOVA ($p < 0.05$) and Pearson correlation analysis. Gene ontological classification was applied to cluster the gene exclusively expressed in DP.

Results: 1) The gene expression profile of DP is markedly different from that of DB and VT (correlation coefficients: DP vs. DB: 0.237; DP vs. VT: 0.08; z-statistics: 12.9, $p < 0.001$); 2) 762 genes were uniquely expressed in DP; 3) Differentially expressed genes for DP are mainly involved in catalytic activity, transport, development, adhesion, transcription factors, differentiation, immune response, cytoskeleton, G-protein-coupled receptors, proliferation, apoptosis and cell motility.

Conclusions: DP is characterized by a unique gene expression profile compared to DB and VT. This finding suggests the topography of the decidua impacts on its gene expression, thereby fulfilling a unique role at the maternal-fetal interface.

435

Effects of Mepyramine and Famotidine on Histamine-Induced Alterations in Human Chorionic Plate Arterial Tone. Mark Wareing, Philip N Baker,[*] Michael J Taggart. *Maternal & Fetal Health Research Centre, The University of Manchester, Manchester, United Kingdom.*

Background: Histamine (HIS) has been suggested to promote placental chorionic plate artery (CPA) relaxation[1,2]. We have demonstrated significant relaxation but only with long term application of agonist[3].

Aim: To determine the pharmacological profile of receptor(s) responsible for the histamine-mediated relaxation of CPAs.

Method: Term placentas (N=8) were obtained post-delivery from uncomplicated pregnancies. Biopsies were placed into ice-cold HCO_3^--buffered physiologic salt solution (PSS). CPAs were dissected, mounted onto a wire myograph, normalised at 0.9 of $L_{5.1kPa}$ and equilibrated (37°C; 20 mins; 5%O_2/5%CO_2). Contraction was assessed with 120mM potassium solution (KPSS). Histamine hydrochloride (1μM for 60 mins) was added to pre-contracted vessels (EC_{80} dose of the thromboxane-mimetic U46619 for 30 mins) in the presence of the H1 receptor antagonist mepyramine (MEP; 3μM), the H2 receptor antagonist famotidine (FAM; 3μM) or both mepyramine and famotidine (MEP+FAM; both 3μM).

Results: Normalised luminal internal diameters were 244±18µm (n=32 arteries). KPSS-induced contraction was 6.6±0.5kPa. HIS produced a triphasic effect. (i) An initial small transient contraction (104±1% of contraction to U46619 at EC_{80} concentration; n=8) abolished by the presence of MEP. (ii) Subsequently, a significant relaxation (to 65±12% of pre-contraction, N=8). Maximal relaxation was significantly attenuated in the presence of FAM (to 91±2%; N=8; P<0.05 ANOVA) and FAM+MEP (to 96±2%; N=8; P<0.05 ANOVA) but not with MEP alone (to 70±10%; N=8; P>0.05 ANOVA). (iii) In the continued presence of HIS, a slight recovery of tone occured such that, after 60 mins, relaxation was 81±12% N=8; P<0.05 t-test). In the presence of FAM+MEP there was no significant HIS-induced relaxation detectable at 60 mins (101±4%; N=8; P>0.05 t-test).

Conclusion: 1µM HIS elicits a complex response; a small transient contraction followed by a biphasic relaxation, in CPAs precontracted with a near-maximal dose of U46619. The initial transient was abolished by H1 receptor blockade whereas H2 receptor blockade was needed to significantly modify the relaxant response. Our data suggest that HIS may play an important role in the control of vascular tone in the fetoplacental circulation.

1. McCarthy *et al* (1994) *AJOG* 171: 1309-15.
2. Sabry *et al* (1995) *Fund Clin Pharm* 9: 46-51.
3. Mills *et al* (2006) *Proc Physiol Soc 3.*
Support: British Heart Foundation.

436

Effects of Hypoxia on Human Placental Veins Investigated under Physiological Conditions with Pressure, Intraluminal Flow and Oxygenation. Mark Wareing, Philip N Baker,* Michael J Taggart. *Maternal & Fetal Health Research Centre, The University of Manchester, Manchester, United Kingdom.*

Background: Hypoxia may increase fetoplacental vascular resistance[1-3]. We have demonstrated that oxygen can modify agonist-induced contraction and relaxation of isolated chorionic plate arteries and veins[4] using wire myography.

Aim: To determine the effect of altered oxygenation on vascular tone in pressurised placental chorionic plate veins.

Method: Term placentas (N=11) were obtained after vaginal delivery or Caesarean section from normal pregnancies. Biopsies were placed into ice-cold HCO_3^--buffered physiologic salt solution (PSS). Isolated human placental chorionic plate veins were equilibrated *in vitro* under conditions of oxygenation (5% O_2/ 5%CO_2 at 37°C); intraluminal pressure (20mmHg) and flow (20µl/min intraluminal flow) designed to mimic an *in vivo* physiological setting. The effects of hypoxia on vascular tone were assessed by switching to 5%CO_2 in N_2. Experiments were repeated in the presence of mild agonist-induced pre-tone (thromboxane-mimetic U46619; $<10^{-8}$M).

Results: Upon completion of the equilibration period, mean luminal diameter of chorionic plate veins was 274±12µm (N=11). Media oxygenation post equilibration was 37.3±0.3 torr (782 oxygen meter; Strathkelvin Instruments). Hypoxia, (pO_2; 2.1±0.1 torr) was accompanied by a small but significant decrease in venous diameter (5.4±0.3% of initial resting diameter in 5% O_2 / 5%CO_2; P<0.05 t-test). U46619 (concentration range 10^{-9}-10^{-8}M) produced a small contraction, as assessed by a 12.4±1.5% decrease in diameter. In the presence of pre-tone, switching to 5%CO_2 / N_2 reduced pO_2 to 1.7±0.1 torr. This reduction in oxygenation produced a further significant decrease in arterial diameter (11.6±1.5% of initial resting diameter in 5% O_2 / 5%CO_2; P<0.05 t-test; P<0.05 paired t-test vs. no tone).

Conclusion: In chorionic plate veins from normal term placentas equilibrated at 20mmHg pressure with intraluminal flow at 5% oxygenation, conditions designed to closely mimic anticipated *in vivo* physiology, subsequent hypoxia elicits vasoconstriction.

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3. Hampl, Bibova, Stranak, Wu, Michelakis, Hashimoto and Archer. (2002). *Am J Physiol (Heart)* 283(6): H2440-2449.
4. Wareing, Greenwood and Baker (2006). *Placenta* 27: 42-48.
Supported by the British Heart Foundation.

437

Gender Differences in the Vascular Reactivity of Chorionic Plate Arteries Following Acute Exposure to Dexamethasone. Justine L Nugent,[1] Rebecca L Jones,[1] Philip N Baker,*[1] Colin P Sibley,*[1] John RG Challis,*[2] Mark Wareing.[1] *[1]Division of Human Development, University of Manchester, Manchester, United Kingdom; [2]Univeristy of Toronto, Toronto, ON, Canada.*

Background: Glucocorticoids play a role in vascular reactivity by increasing vascular resistance via effects on both endothelial and vascular smooth muscle cells. Excess glucocorticoids in pregnancy, either exogenous or endogenous, are associated with intrauterine growth restriction (IUGR). Glucocorticoids, either dexamethasone or betamethasone, are administered in the management of threatened preterm labour. We therefore hypothesized that exposure to dexamethasone alters placental vascular reactivity and thus may contribute to IUGR when administered in pregnancy.

Method: Term placentas were obtained after vaginal delivery or Caesarean section from uncomplicated pregnancies (N = 22 placentas). Chorionic plate arteries were dissected, mounted on a wire myograph, normalised at 0.9 of $L_{5.1kPa}$ and equilibrated for 20mins at 37°C in 5% O_2/ 5% CO_2. Arterial viability was assessed with a 120mMol KCl. Arteries were incubated for 60mins with dexamethasone (10^{-6}M). Arteries were then treated with U46619 (10^{-10}-2x10^{-6}M) and the effect on vasoconstriction was measured. Time controls were performed in parallel.

Results: Normalised luminal diameters of chorionic plate arteries were 327 ± 178µm (n=66 arteries). There was no significant modification in baseline tone. U46619-induced contraction was significantly attenuated by pre-incubation with dexamethasone in placental arteries from female infants (p<0.05; N=10; RM-ANOVA).

Conclusions: These data suggest that the acute exposure of dexamethasone to chorionic plate arteries significantly attenuates vasoconstriction in placentas from female infants only. The exact mechanism behind this action is unknown, but is likely to involve a non-genomic signalling pathway which stimulates endothelial nitric oxide synthase and the release of nitric oxide producing vasodilatation. Dexamethasone is not a substrate for 11β-HSD-2 and therefore it seems unlikely that the gender differences observed result from differential expression of this metabolizing enzyme.

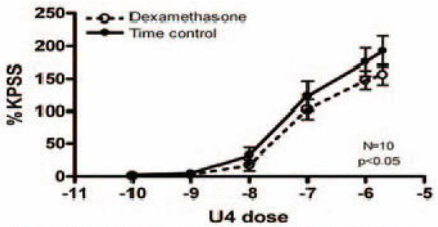

Fig.1: Attenuated vasoconstriction with dexamethasone in chorionic plate arteries - Females.

438

Development of an *In Vitro* Model To Investigate the Effect of Uterine Natural Killer Cells on Vascular Structure. Judith N Bulmer,[1] Gendie E Lash,[2] Katsuhiko Naruse,[2] Barbara A Innes,[2] Harry A Otun,[2] Roger F Searle,[3] Stephen C Robson.*[2] *[1]CALS, Newcastle University, Newcastle, United Kingdom; [2]SARS, Newcastle University, Newcastle, United Kingdom; [3]MED, Newcastle University, Newcastle, United Kingdom.*

Background: Remodeling of uterine spiral arteries (SpA) is essential for successful human placentation. Research to date has focused on the role of trophoblast in this process, although morphological alterations are seen in SpA before tropohoblast invasion. Uterine natural killer (uNK) cells appear to be essential for arterial modification in mouse pregnancy, mediated by interferon-γ. Several angiogenic growth factors are produced by human uNK cells but evidence of a role for uNK cells in priming of SpA prior to trophoblast invasion in human pregnancy is circumstantial. We propose that uNK cells cause alterations in the media of uterine SpA faciltating subsequent invasion by extravillous trophoblast.

Objective: To develop a simple *in vitro* model to allow investigation of the role of uNK cells in priming of uterine SpA in early pregnancy and the underlying mechanisms.

Methods: Chorionic artery segments were dissected from normal term placentas. Intact cross sections 5mm length were incubated in medium alone or in medium containing 20% (vol/vol) supernatant harvested after 24h culture of uNK cells (10-12 weeks gestational age) purified by immunomagnetic positive selection (CD56+). Chorionic arteries were harvested after 5 days, formalin fixed and paraffin embedded (n=5). 3µm sections were immunostained for

FRIDAY

h-caldesmon and myosin heavy chain. Immunoreactivity of medial smooth muscle was analysed using an Adobe Photoshop-based technique. Protein was extracted from other chorionic artery samples (n=3) for Western analysis of connexins.

Results: After 5 days culture control samples showed intact structure and endothelial preservation. There was increased separation of chorionic artery smooth muscle after incubation in uNK cell supernatant compared with controls (surface area of h-caldesmon positive immunostaining: controls 49.6±8.1%; uNK cell supernatant 26.9±8.2%; P=0.016). Western analysis showed reduced connexin 43 in samples incubated with uNK cell supernatant compared with controls.

Conclusions: Soluble products of uNK cells induce structural changes in an *in vitro* model. Chorionic artery rings provide a useful, simple and readily available *in vitro* model to investigate the effect of uNK cells on arterial structure which complements *in situ* studies.

439

Isolation of Fetal Microvascular Endothelial Cells and Subsequent RNA Extraction from Normal and Preterm Placenta. Anne M Roggensack,[1] Caroline E Dunk,[2] S Lee Adamson,[*3] John Kingdom.[*1] *[1]Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [3]Obstetrics and Gynecology, Pediatrics, and Physiology, Mount Sinai Hospital, Toronto, ON, Canada.*

The physiology of the placental vasculature is unlike any other human organ. As endothelial cells isolated from different vascular beds exhibit unique gene expression profiles, this concept likely also applies to placental microvascular endothelial cells due to their unique role in placental development and function. However, fetal microvascular endothelial cells and their RNA, as yet, have not been successfully isolated from placenta. Thus, this important vascular compartment has not yet been well studied. Our objective was to develop a unique protocol to isolate a pure solution of fetal microvascular endothelial cells and extract RNA. Random 50g samples were obtained from 3 normal term and 6 preterm placentas. The outer 2/3 of placenta was sampled (i.e. closest to the maternal surface), to preferentially sample the terminal villi. Fresh placental tissue was minced, and then digested with trypsin, separating trophoblast cells away from the microvasculature. The remaining tissue was further digested with collagenase, separating the cells into suspension. Blood cells were removed from the suspension by gradient separation with Ficoll. Cell viability was confirmed using Trypan blue exclusion. Tocosylated magnetic Dynabeads, labeled with Human Endothelial Antigen (HEA) lectin (using the method of Gallery et al), were administered to the suspension, positively selecting intact endothelial cells. The purity of the endothelial cells collected was assessed by CD31 immunocytochemistry. RNA was extracted immediately, purified using Trizol, and stored at -80°C. This novel protocol has been successful in isolating 95% pure microvascular endothelial cells, with between 22.8µg - 110.3µg of RNA extracted per 50g of placental sample. The RNA collected was of high quality, and therefore suitable for microarray, RT-PCR, and/or Northern blot. This novel method successfully isolates a previously difficult to access cell type, and will lead to advances in our understanding of gene expression in fetal microvascular endothelial cells in normal and pathologic pregnancies. This work is supported by the MFM Fellow's Research Fund, Mount Sinai Hospital.

440

Vitamin C and E Supplementation Improves Placental Vessel Function in Women at Risk of Pre-Eclampsia. Tracey A Mills,[1] Mark Wareing,[1] Susan L Greenwood,[1] Lucilla Poston,[*2] Andrew H Shennan,[2] Philip N Baker.[*1] *[1]Division of Human Development, The University of Manchester, Manchester, United Kingdom; [2]Maternal and Fetal Research Unit, Kings College London, London, United Kingdom.*

Background: Pre-eclampsia is associated with oxidative stress. The vitamins in pre-eclampsia (VIP) trial [1] assessed whether maternal vitamin C and E supplementation could reduce pre-eclampsia (PE) in women at risk. Increased fetoplacental vascular resistance, a complication of PE, might arise from inappropriate vessel reactivity secondary to chronic oxidative stress and could be alleviated by antioxidants in women at risk.

Aim: To investigate the effect of vitamin C and E supplementation on placental chorionic plate artery function in a cohort of VIP trial participants.

Methods: Post-delivery, chorionic plate arteries from women randomized to antioxidants (N=15) or placebo (N=21) were prepared for wire myography. Arterial contractility to U46619 (thromboxane A_2 mimetic; 10^{-10}-10^{-6}M) was determined. Paired vessels were incubated with xanthine (XA;10^{-4}M) plus xanthine oxidase (XO;10mU/ml), to generate reactive oxygen species or vehicle diluent during the remaining experiment. After 10 min, the U46619 dose-response curve was repeated. Endothelium-independent relaxation was assessed in pre-constricted vessels (U46619 EC_{80}) using sodium nitroprusside (SNP; NO donor, 10^{-9}–10^{-4}M).

Results: Maximal contraction was attenuated by antioxidant supplementation (antioxidant v placebo; 7.6±0.7kPa vs. 9.9±0.8kPa, mean±SEM P<0.05, M-W U test) but the EC_{50} was unaffected (antioxidant vs. placebo; 64±13nM vs. 39±8nM). Supplementation did not affect relaxation to SNP compared to placebo. XA/XO enhanced basal tone and contraction to U46619 in both vitamin and placebo groups. XA/XO enhanced relaxation to SNP in the placebo group only (maximum relaxation to 43±5% vs. control relaxation to 55±6% of U46619 EC_{80} constriction with 10^{-5}M SNP; P<0.05 2-way ANOVA).

Conclusion: Vitamin C and E supplements improved the vascular reactivity of chorionic plate arteries in women at risk of PE. We propose that antioxidant supplementation might improve placental vascular function and blood flow *in-vivo*. The VIP trial demonstrated that prophylactic antioxidant treatment did not reduce the incidence of PE in women at risk [1]. These data suggest that oxidative stress effects on the placental vasculature are not the major cause of PE.

1.Poston, Briley, Seed, Kelly & Shennan AH (2006) *Lancet*; 367:1145-1154.

441

The Human Placenta Is an Extra-Hepatic Source of Vitamin K-Dependent Anti-Coagulant Proteins. Chong Jai Kim,[1,2] Roberto Romero,[*1,3] Yu Mi Han,[1] Sung-Su Kim,[1] John Hotra,[1] Jung-Sun Kim,[1] Francesca Gotsch,[1] Offer Erez,[1] Juan Pedro Kusanovic,[1] Jimmy Espinoza.[1,4] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Pathology, Wayne State University, Detroit, MI, USA; [3]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Objective: Pregnancy is characterized by increased generation of thrombin and is a hypercoagulable state. The balance between pro- and anti-coagulant factors is particularly important in the intervillous space of the placenta, where both prevention of thrombosis and efficient hemostasis are required. This study was conducted to determine whether the placenta and its cellular compartments express subsets of vitamin K-dependent, anti-coagulant plasma proteins: protein C (PC), protein S (PS), protein Z (PZ). The conventional view has been that these proteins are produced by the liver.

Methods: The expression of PC, PS, and PZ mRNA and proteins were studied in preterm (n=9) and term placentas (n=24). Expression was also screened in HTR-8/SVneo and JAR cell lines. mRNA in situ hybridization, qRT-PCR, immunohistochemistry, and immunoblotting were used for analyses.

Results: 1) PC, PS, and PZ mRNA and protein expression was detected in the placenta and fetal membranes by mRNA in situ hybridization, immunohistochemistry, and immunoblotting; 2) Extravillous and villous trophoblasts were strongly immunoreactive to PC and PZ; 3) Amnion cells showed distinct immunoreactivity to PC and PZ; 4) In contrast, trophoblasts and amnion cells were weakly reactive to PS. However, PS mRNA expression was relatively abundant compared to that of PC and PZ; 5) PC, PS, and PZ mRNA and protein were also detected in HTR-8/SVneo and JAR trophoblast cells; and 6) Maternal decidua did not show immunoreactivity to PC, PS, and PZ.

Conclusion: 1) The human placenta is a novel extra-hepatic source of anti-coagulant proteins PC, PS, and PZ; 2) The expression of PC, PS, and PZ by trophoblasts, but not in decidua, suggests compartmentalization of anti-coagulant factors during pregnancy; 3) The lower expression of PS protein, but not mRNA in trophoblasts, suggests that a post-transcriptional regulatory mechanism is operative; and 4) We propose that the expression of anti-coagulant proteins by villous trophoblast is central to the prevention of thrombosis in the intervillous space.

442

Is a Change in Placental VEGF and Flt-1 Expression a Sign of Vascular Regeneration? Thushari I Alahakoon,[1] Susan Arbuckle,[2] Weiyi Zhang,[1] Brian J Trudinger.*[1] [1]*Obstetrics and Gynecology, University of Sydney at Westmead Hospital, Westmead, NSW, Australia;* [2]*Anatomical Pathology, New Childrens Hospital, Westmead, NSW, Australia.*

Objective

Placental vascular disease (PVD) can be recognized in the antenatal period by a high resistance pattern in the umbilical artery Doppler (UAD) flow velocity waveforms. It is associated with intrauterine growth restriction (IUGR). Vascular endothelial growth factor (VEGF) has been attributed a major role in the mediation of vasculogenesis and angiogenesis. The aim of this study was to determine the expression and localization of placental VEGF and its receptor Flt-1 in pregnancies complicated by PVD with and without pre-eclampsia.

Methods

Placentas were studies from four groups of patients. Group1: uncomplicated term pregnancies (n=5); group 2: pre-eclampsia with normal UAD (n=9); group 3: pre-eclampsia with abnormal UAD and IUGR (n=6); group 4: IUGR with abnormal UAD (n=10). 4 placental samples were examined from each pregnancy. Immuno-histochemical staining intensity for VEGF and Flt-1 was assessed by an independent pathologist and correlated with histopathological changes.

Results

The expression of VEGF and Flt-1 correlated with histological changes, rather than with the clinical groups. The placentas from groups 2-4 displayed focal areas of villous infarction. The residual villi within samples containing infarcted villi, demonstrated reduced VEGF immuno-reactivity in the syncytiotrophoblast ($p<0.001$). Placental samples without evidence of villous infarction demonstrated increased VEGF expression in syncytiotrophoblast ($p<0.0001$). Flt-1 expression was significantly increased in all samples from groups 2-4. ($p<0.001$).

Conclusion

We have correlated the angiogenic response of VEGF and Flt-1 in the placenta to histopathological changes as well as clinical features of UAD waveforms and pre-eclampsia. Placental vascular disease is a multifocal disease of the placenta with evidence of villous infarction. We have demonstrated that villi in the peri-infarction areas express reduced VEGF while rest of the placenta shows a compensatory increase in VEGF and Flt-1. These responses may reflect a placental repair or regeneration process to restore the vascularity in face of an insult. The results of our study raise the possibility that these changes in VEGF and Flt-1 expression may be a consequence rather than the cause of placental vascular disease and pre-eclampsia.

443

Abstract Withdrawn.

444

Abstract Withdrawn.

445

Why Does Umbilical Phlebitis Precede Arteritis in Funisitis? Jung-Sun Kim,[1] Roberto Romero,*[1,2] Christopher LaJeunesse,[3] Yeon Mee Kim,[1,3] Pooja Mittal,[1,4] Jimmy Espinoza,[1,4] Chong Jai Kim.[1,3] [1]*Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA;* [2]*Center of Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA;* [3]*Department of Pathology, Wayne State Univeristy, Detroit, MI, USA;* [4]*Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Objective: Acute funisitis is the histologic hallmark of the 'fetal inflammatory response syndrome' (FIRS), which is a risk factor for preterm delivery. A key feature of funisitis is umbilical vasculitis. Phlebitis invariably precedes arteritis, marking different stages of FIRS. Yet, the mechanism for the increased susceptibility of the umbilical vein (UV) for leukocyte infiltration is unknown. This study was conducted to determine if there is a difference in the proinflammatory response between the UV and umbilical artery (UA) in patients with and without funisitis.

Methods: Segments of UV and UA were collected from the following groups: 1) normal pregnancy at term not in labor (n=12); 2) normal pregnancy at term in labor (n=12); 3) preterm delivery (PTD) with and without histologic chorioamnionitis (n=11 and n=14, respectively). mRNA expression of interleukin-1β (IL-1β) and IL-8 was evaluated by qRT-PCR. Explant cultures from 5 sets of UV and UA from normal pregnancies at term were used to study the regulation patterns of cytokines following lipopolysaccharide (LPS) treatment in vitro. Non-parametric statistics were used.

Results: 1) mRNA expression of IL-1β and IL-8 was higher in UV than in UA in women at term not in labor ($p=0.006$ and $p=0.015$, respectively), as well as those in labor ($p=0.002$ and $p=0.003$, respectively); 2) IL-8 mRNA expression in UV and UA of women at term in labor was higher than those patients with PTD without chorioamnionitis ($p=0.027$ and $p=0.042$, respectively); 3) mRNA expression of IL-1β in UA, and IL-8 in UV and UA from patients with PTD with chorioamnionitis was high when compared to patients with PTD without chorioamnionitis ($p=0.003$, $p=0.016$, and $p=0.018$, respectively); 4) LPS increased mRNA expression of IL-1β and IL-8 in both UA and UV ($p=0.043$).

Conclusions: 1) The UV displays a higher proinflammatory response than the UA in term gestations; 2) IL-8 mRNA expression increases as a function of gestational age in the absence of inflammation in both UV and UA. We propose that these novel findings explain why umbilical phlebitis precedes arteritis in the context of funisitis.

446

Roles of Protein Phosphatase 2B (PP2B) in Regulating FGF2- and VEGF-Stimulated Cell Proliferation in Ovine Feto-Placental Artery Endothelial (OFPAE) Cells. Kai Wang, Yang Song, Jing Zheng.* *Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA.*

Reversible protein phosphorylation is a critical process for regulating cellular function. This process is tightly controlled by protein kinases (i.e., ERK1/2) and protein phosphatases (i.e., PP2B, a serine/threonine protein phosphatase). Recently it has been reported that inhibition of PP2B in human umbilical vein endothelial cells prevents VEGF-induced angiogenesis. We have observed that in OFPAE cells inhibition of PP2A does not affect FGF2- and VEGF-stimulated cell proliferation. In this study, we tested whether suppression of PP2B enhanced FGF2- and VEGF-stimulated cell proliferation and NOS3 phosphorylation partly via activation the MEK/ERK1/2 signaling pathway in OFPAE cells. **Methods:** Small interfering RNA (siRNA) specifically targeting PP2B catalytic subunit α ($PP2Bc_\alpha$) was used to suppress $PP2Bc_\alpha$ expression in OFPAE cells. Cell proliferation was assayed with crystal violet method. Levels of ERK1/2 and NOS3 were determined by Western blot analysis. **Results:** Transient transfection (2 days) of $PP2Bc_\alpha$ siRNA decreased ($p<0.05$) protein levels of $PP2Bc_\alpha$ and total NOS3 by ~ 90% and ~ 40%, respectively, as compared with the scrambled siRNA, but did not alter $PP2Ac_\alpha$ and GAPDH protein levels. This suppression of $PP2Bc_\alpha$ expression maintained at least for 3 days after transfection. Suppression of $PP2Bc_\alpha$ expression appeared to promote VEGF-, but not FGF2-induced cell proliferation, and increased FGF2-, but not VEGF-stimulated NOS3 phosphorylation on Ser1177. Moreover, the suppressed $PP2Bc_\alpha$ expression was associated with an increase in VEGF-induced ERK1 phosphorylation and a decrease in FGF2-induced ERK1/2 phosphorylation. **Conclusions:** These data suggest that in OFPAE cells suppression of PP2B promotes VEGF-induced cell proliferation and FGF2-stimulated NOS3 phosphorylation partially via increasing and decreasing ERK1/2 activity, respectively. (Supported by NIH grants HL64703 to JZ).

447

Blockage of Fibroblast Growth Factor Receptor 1 (FGFR1) Disrupts Ovarian Function through Its Effects on the Hypothalamus-Pituitary (H-P) Axis and Not Disrupting Ovarian Angiogenesis Locally. Raul Gomez,[1] Hongyan Tang,[1] Nataki C Douglas,[1] Mark V Sauer,*[1] Haijun Sun,[2] Ralf C Zimmermann.*[1] [1]*Ob&Gyn, Columbia University, New York, NY, USA;* [2]*Imclone Systems Incorporated, New York, NY, USA.*

Objectives

FGFR1 signaling regulates cell proliferation, migration and survival being essential for various stages of mammal development. High ovarian mRNA expression levels and upregulation during luteal phase suggested FGFR1 might (as VEGFR2) be a main regulator of ovarian function and angiogenesis locally. Recently, the hypogonadotropic hypogonadism has been linked to mutations in this receptor suggesting FGFR1 could regulate ovarian function at the H-P level. To answer this question IMC-A1 (Imclone FGFR1 blocking antibody) was administered at a 10 mg/kg dose to female CD1 mice using different approaches

Design:

Three experimets were performed

1) Mice (n=5) were injected i.p at the estrous stage (day 0) with IMC-A1. Control animals (n=5) received unspecific IgG). Injections were continued in both groups every 48 hours until day 12 when animals were euthanized

FRIDAY

2) Conditions identical to set 1 plus follicle stimulation with PMSG 20 IU on day 12. Animals were killed 48 hours later (day 14)
3) Design identical to set 2, plus ovulation induced with hCG 20 IU on day 14 (also injected with IMC-A1 at this point) animals were killed 24 hours later (day 15)

Methods

After euthanization ovaries and uteri were dissected, weighed and fixed for IHC. Circulating P4 levels were measured. T-test was performed

Results

FGFR1 blockage suppressed ovarian function. No corpora lutea (CL), preovulatory or antral follicles were observed in IMC-A1 treated animals. Ovarian and uterus weights were decreased by 3 and 6 fold respectively vs controls, $p<0.01$. PMSG restored folliculogenesis in IMC-A1 treated mice. Similar ovarian and uterus weights and number of preovulatory and antral follicles in control and treatment groups were observed. HCG induced CL formation in IMC-A1 treated mice. Equal number of corpora lutea (8-12), ovarian or uterine weights were found in both groups

Discussion:

FGFR1 blockage suppressed ovarian function. Exogenous gonadotropins restored ovarian function in IMC-A1 treated animals. In addition, P4 decreases (a subsequent effect of disrupted luteal angiogenesis) were NOT observed in PMSG+hCG animals treated with IMC-A1. We conclude that FGFR1 signaling plays a critical role regulating ovarian function, not locally, but centrally through the H-P axis.

448

Calcitonin Gene-Related Peptide (CGRP) Is a Pro-Angiogenic Growth Factor in the Human Placental Development. Yuan L Dong,* Deepti M Reddy, Xin Ma, Hui-Qun Wang, Manubai Nagamani,* Gary DV Hankins,* Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

BACKGROUND: Recent studies have shown that homozygous knockouts of gene for CGRP receptor component, calcitonin receptor-like receptor (CRLR) led to defective placental vasculature and embryonic death, underlining the critical role of CGRP in placental development and fetal growth.

OBJECTIVE: To determine: 1) expressions of CGRP and its receptor components (CRLR/$RAMP_1$) at the human implantation site during early pregnancy; 2) whether CGRP regulates *in vitro* angiogenesis of human umbilical vein endothelial cells (HUVEC); and 3) if CGRP can improve angiogenic imbalance in preeclamptic placental explants.

METHODS: Placental tissues were obtained either during legal first-trimester termination or during the Cesarean section in normal term pregnancies and those with preeclampsia. Cellular localization of CGRP and its receptor components were determined using immunofluorescent confocal imaging analysis. Pro-angiogenic bio-activity of CGRP was evaluated using HUVEC migration and capillary-like tube formation on the Matrigel.

RESULTS: 1) Immunoreactive CGRP is localized primarily in cytoplasm of villous cytotrophoblasts and syncytiotrophoblasts, as well as extravillous trophoblast cells in the interstitial tissues; 2) CGRP receptor components, CRLR and $RAMP_1$, are expressed by both villous and extravillous trophoblast cells, as well as vascular endothelial cells identified by endothelial cell marker CD_{34} antibodies; 3) treatment with CGRP (10^{-8} M) for 12 h significantly increased HUVEC migration on Matrigel ($150 \pm 11\%$ vs. $115 \pm 5\%$, $p < 0.01$) and capillary-like tube formation ($211.3 \pm 21.5\%$ vs. $121 \pm 6\%$, $p < 0.01$); 4) increases in both HUVEC migration and capillary-like tube formation are completely blocked by CGRP antagonist, $CGRP_{8-37}$ (10^{-7} M); and 5) culture medium from preeclamptic placental explants significantly inhibits HUVEC capillary-like tube formation compared to gestation age-matched control ($38 \pm 4\%$ vs. $100 \pm 6\%$, $p < 0.01$); and 7) culture medium from preeclamptic placental explants incubated with CGRP (10^{-7} M for 48h) significantly improved placental angiogenesis evidenced by increased capillary-like tube formation ($79 \pm 5\%$ vs. $38 \pm 4\%$, $p < 0.01$).

CONCLUSION: CGRP at the human implantation site may constitute a potential autocrine or paracrine mechanism that could modify placental angiogenesis and neovascularization.

449

Strategic Localization of CRF, CRF-Receptors and CRF-Binding Protein in Human Term Umbilical Cord. Jayaraman Lakshmanan,[1] Michael G Ross,*[1] Chander P Arora,[2] Lilit Baldjyan,[2] Boudaie Alex,[2] Vignesh Arasu,[2] Guo L Liu,[1] Calvin J Hobel.*[2] *[1]Obstetrics & Gynecology, Harbor-UCLA Medical Center, Torrance, CA, USA; [2]Obstetrics & Gynecology, Cedars-Sinai Medical Center, Burns & Allen Research Institute, Los Angeles, CA, USA.*

Objective: To examine the key molecules of the CRF system in term human umbilical cord.

Hypothesis: The expression of CRF, CRF-receptors and CRF-BP within the umbilical cord should support a potential cellular mechanism for their vascular functions.

Introduction: Corticotrophin releasing factor (CRF) regulates multiple functions in feto-placental unit. CRF mediates its biological effects through two major types of receptors, CRF-receptor type-1(CRF-1) and CRF-receptor type 2 (CRF-R2). CRF-binding (CRF-BP) protein, a key modulator of CRF actions, has long been speculated to compete with both receptors for CRF.

Study Design: Umbilical cords collected at delivery after uncomplicated full-term pregnancy (n=6) were cut (3-4mm thickness), fixed in Bouin's solution, and processed for paraffin embedding. Five micron paraffin sections were subjected to immunohistochemistry by avidin-biotin-complex system with rabbit polyclonal antibody to rhCRF (1:300, Peninsula Laboratory) or goat polyclonal antibody that specifically interacts with both CRF-R1 and CRF-R2 receptors (1:200, Santa Cruz) as well as with rabbit polyclonal antibody to CRF-BP (1:200, Santa Cruz biotechnology, Inc). Immunoreactive materials on the sections were identified as brown staining using 3', 3'diaminobenzadine as a chromogen. Sections incubated with normal rabbit or goat IgG were used as appropriate controls.

Results: The CRF antibody very strongly immunostained Wharton Jelly cells while both CRF-R1/R2 receptors and CRF-BP immunostained smooth muscle cells.

Conclusion: We interpret the strong positive staining observed with CRF antibody in Wharton Jelly cells as evidence that these cells are the site of CRF expression. The findings of CRF-R1/R2 receptor immunostaining in vascular smooth muscles cells imply that these receptors regulate vasomotor functions of the umbilical cord allowing maximal feto-placental perfusion near parturition. The staining pattern observed with CRF-BP antibody in smooth muscle cells suggests a regulatory role for this protein at the level of vascular smooth muscle cells. In summary the cellular localization of CRF, CRF-Receptors and CRF-BP protein in the umbilical cord are strategically localized and functionally coordinated.

450

Identification of a Transcriptional Inhibitory Motif in the Human Placental Growth Factor (PlGF) Gene Promoter. Christophe L Depoix,[1] Meng Kian Tee,[2] Robert N Taylor.*[1] *[1]Gynecology and Obstetrics, Emory University, Atlanta, GA, USA; [2]Pediatric Endocrinology, University of California, San Francisco, San Francisco, CA, USA.*

Objective: Preeclampsia is a syndrome characterized by abnormal placental angiogenesis manifested as early as the midtrimester of pregnancy. We hypothesized that this defect was due, in part, to an inhibition of PlGF gene transcription by trophoblasts under hypoxic conditions.

Methods: The regulation of PlGF expression was studied using real-time quantitative reverse transcription PCR (qRT-PCR) and ELISA in JEG-3 and BeWo choriocarcinoma cell models of human trophoblasts. Cells were treated with cobalt chloride to mimic hypoxia. To systematically search for inhibitory elements in the human PlGF gene promoter we cloned genomic DNA extending from -3450 bp to +1 bp relative to the transcriptional start site into a promoterless luciferase vector and created a series of deletion constructs. The constructs were then transiently transfected into the choriocarcinoma cells and analyzed.

Results: Cobalt chloride dose-dependently reduced steady-state levels of PlGF mRNA to less than 20% of control and PlGF protein also was suppressed. Our transfection results revealed that an element(s) located between -3450 bp and -2141 bp conferred transcriptional repression of PlGF-luciferase plasmids in both cell types. Constructs containing this motif suppressed luciferase activation by 90%, approaching the activity of an empty reporter vector. Deletion of this 1309 bp sequence restored full transcriptional activity of the promoter.

Computational analyses of the inhibitory region revealed several potential cis-acting motifs with reported repressor activity, including Ying-Yang 1 repressor sequences and GATA factor binding sites. Interestingly, consensus hypoxia- and metal responsive elements believed to mediate hypoxic repression of the PlGF gene in trophoblasts are located in the proximal -860 bp of the gene promoter, distinct from the identified 1309 bp motif.
Conclusions: We postulate that, in addition to hypoxic effects, decreased expression of PlGF in preeclamptic women may be due to the over-expression of other transcription factors binding to inhibitory motifs or secondary to gene polymorphisms within this upstream sequence. Experiments are in progress to map the elements and to isolate the regulatory factors that bind to them.
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451

Elastin Breakdown: The Key to Successful Spiral Artery Remodelling?
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Mural remodelling of the myometrial spiral arteries during pregnancy to generate high flow, low resistance channels requires breakdown of extracellular matrix components within the vessel wall, including the internal elastic lamina (IEL) and elastin fibres within the arterial media. Breaks in the IEL are apparent in placental bed biopsies, yet the elastases involved in this process are currently unknown. Breakdown of elastin is impaired in the myometrial segments of spiral arteries in pre-eclampsia. **Objective:** To determine the origin and identity of the elastases that mediate transformation of the spiral arteries. **Methods:** The intracellular elastase activity of primary first trimester cytotrophoblasts (CTB) and vascular smooth muscle cells (SMC) was detected using a cell-permeable fluorescent elastase substrate. Total elastase activity of cell lysates prepared using Triton-X100 was measured using the substrate N-succinyl-(L-alanine)$_3$-p-nitroanilide, and compared to a standard curve prepared using porcine pancreatic elastase. Immunohistochemistry was performed using antibodies against MMP-12 and MMP-14. **Results:** CTB engulfed and degraded elastin fibres and exhibited intracellular elastase activity. CTB lysates also exhibited elastase activity, which was inhibited by a broad spectrum matrix metalloprotease (MMP) inhibitor. A transcriptomic database and immunohistochemistry identified MMP-14 as a candidate CTB elastase. Vascular SMC exhibited little intracellular elastase activity in vitro; however, the presence of elastin fragments elevated this activity 6.3 fold ($p \leq 0.05$). SMC lysates demonstrated elastase activity, which was inhibited by a broad spectrum MMP inhibitor. Immunohistochemical studies implicated MMP-12 and MMP-14 as candidate SMC elastases. Expression of MMP-12 was increased following treatment with trophoblast conditioned medium in SMC cultured in vitro or in situ. **Conclusion:** Trophoblasts utilise membrane-bound and intracellular elastases to effect a regulated pericellular degradation of elastin. As elastase activity in SMC is stimulated by elastin fragments and soluble factors secreted by trophoblasts, the process of trophoblast invasion may induce cooperative elastin breakdown by resident medial SMC.

452

Integrin Linked Kinase (ILK) in Human Endometrial Endothelial Cells; Implication for Endometrial Angiogenesis and Angiostasis.
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Objective: Human endometrium shows temporal and spatial changes in integrin expression throughout the menstrual cycle and early pregnancy. Integrin-linked kinase (ILK) is a key cytoplasmic serine/threonine protein kinase involved in a range of signaling pathways and functions as a scaffold by mediating interactions between integrins and the actin cytoskeleton. ILK is involved in vascular development and endothelial cell survival, and is a regulator of angiogenesis. We hypothesized that ILK exhibits changes in its expression pattern in human endometrial endothelial cells throughout the menstrual cycle in parallel to angiogenic changes in endometrium.
Materials and methods: Immunohistochemical staining was performed using anti-human ILK antibody in 33 endometrial tissues from normal women without endometrial disease and in 5 decidual tissues from women with clinically normal pregnancies terminated voluntarily during the first trimester. ILK staining was evaluated with using HSCORE according to menstrual cycle phases. Statistical analyses of the data was assessed by one-way ANOVA, with $p<0.05$ considered significant.
Results: ILK immunoreactivity was observed mainly in stromal and endothelial cells. HSCORE analysis showed that ILK expression (284±9) does not change significantly except for the endothelial cells (169±19) from mid-secretory endometrial tissues ($p<0.05$). On the other hand, endothelial cells of spiral arteries revealed a significant decrease (120±12) in ILK immunoreactivity in early pregnancy decidual tissues when compared with microvascular endothelial cells from the same samples and from the other menstrual cycle phases ($p<0.05$).
Conclusion: Strong ILK expression in human endometrial endothelial cells supports the idea that ILK is involved in endometrial angiogenesis. Furthermore, decrease in ILK level in endothelial cells during implantation window and in spiral arteries in early pregnancy decidual tissues may facilitate intersitial cytotrophoblasts invasion into maternal vessels.

453

Effect of Smoking on Circulating Angiogenic Factors in High Risk Pregnancies.
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Objective: Alterations in maternal circulating concentrations of the antiangiogenic factors, soluble fms-like tyrosine kinase 1 (sFlt1) and endoglin, and pro-angiogenic placental growth factor (PlGF) precede the development of preeclampsia in healthy low-risk women. Smoking during pregnancy is associated with reduced risk of preeclampsia, reduced sFlt-1 and endoglin, as well as increased PlGF in low-risk women. The objective of this study was to investigate whether smoking affects angiogenic factors (sFlt-1, PlGF, and endoglin) in women at high risk for developing preeclampsia.
Study Design: We performed a secondary analysis of serum samples from 993 high-risk women in the NICHD MFMU aspirin trial to prevent preeclampsia. sFlt1, endoglin and PlGF were measured in serum samples obtained at randomization and prior to initiation of aspirin (mean 19.3, range of 7.6-26.9 weeks). Smoking status was determined by self-report. Data are presented as mean ± standard deviation and analyzed by Wilcoxon rank sum test.
Results: At baseline, cigarette smoking was not associated with altered levels of sFlt-1 in any of the high-risk groups. Circulating PlGF was higher in pregnant smokers with diabetes compared with nonsmokers with diabetes ($p=0.005$) and in pregnant smokers with previous preeclampsia ($p=0.001$). Lower concentrations of circulating soluble endoglin were observed at baseline in smokers with multifetal gestations ($p=0.002$). Smoking was not associated with a reduction in preeclampsia in any of these groups.

	Pregestational diabetes		Chronic hypertension	
	Nonsmoker (n=155)	Smoker (n=39)	Nonsmoker (n=260)	Smoker (n=53)
sFlt1 (ng/ml)	3.7±2.2	3.5±1.6	3.7±3.7	3.7±2.3
Endoglin (ng/ml)	5.5±1.8	5.0±1.6	5.8±5.7	5.2±2.0
PlGF (pg/ml)	135.6±126.1	219.7±190.8*	208.4±209.7	246.6±261.7
	Multifetal Gestation		Previous preeclampsia	
	Nonsmoker (n=203)	Smoker (n=31)	Nonsmoker (n=212)	Smoker (n=40)
sFlt1 (ng/ml)	6.3±3.3	5.4±2.3	3.4±1.9	2.8±1.1
Endoglin (ng/ml)	7.5±3.2	5.8±1.4*	5.3±2.3	6.8±10.2
PlGF (pg/ml)	515.9±375.0	596.8±424.6	204.3±192.8	327.4±258.6*

* $p < 0.05$

Conclusions: In certain high-risk groups, smoking is associated with changes in the circulating angiogenic milieu in early pregnancy that could influence the development of preeclampsia in later gestation.

454

Local Cortisol Availability Influences Endometrial Angiogenesis.
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Introduction Heavy Menstrual Bleeding (HMB) is common, requiring drug therapy or surgery. We studied the endometrium of women with HMB, focussing on glucocorticoid metabolism, since glucocorticoids modulate angiogenesis in other organs.
Hypothesis Endometrial glucocorticoid availability may modulate angiogenesis and thus impact upon menstrual blood loss.

FRIDAY

Methods Endometrial tissue was collected from 29 women with menstrual complaints. Subjects provided written informed consent and studies had Institutional ethical approval. Biopsies were consistent for histological stage, LMP, and serum sex steroid levels at time of biopsy. Menstrual blood loss (MBL) was measured by alkaline-haematin assay, (>80ml MBL as indicative of HMB). qRT-PCR for angiogenic marker genes (angopoietin-1 (ANG-1) and -2 (ANG-2); vascular endothelial growth factor-A (VEGF-A); thrombospondin-1 (TSP-1) and glucocorticoid metabolising enzymes 11βhydroxysteroid dehydrogenases -1 and -2 (11βHSD1,2) was performed. *De novo* angiogenesis was assessed in 3D-culture, and endometrial biopsies provided stromal cells (ESC) to determine effects of glucocorticoids on angiogenic signalling. Endothelial cell tube forming assays in the presence of cortisol were performed.

Results In the secretory phase, increased expression of 11βHSD2 and ANG-1, and decreased expression of TSP-1 mRNA was observed when MBL>80ml (heavy MBL; HMBL) as compared to MBL<80 ml (P<0.05). 11βHSD2 was immunolocalised to epithelial cells (glandular and surface), and, consistent with mRNA expression showed increased immunoreactvity in the HMBL samples. ANG-2, VEGF-A and 11βHSD1 mRNA were unaffected by MBL status. *In vitro*, cortisol administration (1μM) significantly decreased endothelial sprouting (P<0.05), an effect replicated by 1nM TSP-1. ESC responded to cortisol treatment by increased mRNA expression of TSP-1 (P<0.05). Uterine endothelial tube forming was dose-dependently inhibited by cortisol (P<0.01).

Conclusions Increased 11βHSD2 expression predicts reduced local availability of cortisol in HMBL patients. Cortisol is likely important for the expression of anti-angiogenic TSP-1 and regulation of *de novo* angiogeneis. Glucocorticoids may exert control over anti-angiogeneic factors in endometrium, interruption of this by abberant expression of glucocorticoid metabolising enzymes may contribute to abnormal menstrual bleeding.

455

Immunolocalization of Intermedin in Human Placenta: A Novel Angiogenic Role of IMD in Placenta. Madhu S Chauhan, Uma Yallampalli, Yuan-Lin Dong,* Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Intermedin (IMD)/Adrenomedullin (AM) 2 is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) family. In this group of peptides IMD and adrenomedullin (AM) share the highest structural homology. Recently we demonstrated that similar to AM, IMD antagonist (IMD_{17-47}) causes feto-placental growth restriction, apoptotic changes and impairment of placental morphology in placenta of IMD_{17-47} infused rat. Therefore, we hypothesize that IMD is expressed in human placenta and is involved in placental angiogenesis.

OBJECTIVES: 1) To immunolocalize IMD protein in the first, second and third trimester human placenta; 2) To assess the effects of IMD peptide on trophoblast invasion in HTR-8SV/neo cells; and 3) to assess the effect of IMD peptide on the expression of angiogenic agents such as VEGF and flt-1 in first trimester HTR-8/SVneo cells.

METHODS: The consent form and procedure for this study were approved by the institutional research board of UTMB. Samples of placental villous tissues were obtained from normal third trimester pregnant women or from elective cesarean sections of the first and second trimester pregnant women. Five μm thick tissue sections were used in immunofluorescent studies for double labeling using CD34, CK7 and IMD specific antibodies. First trimester HTR-8SV/neo cells were also used to assess the effect of IMD (10^{-6} M and 10^{-7}M) on trophoblast cell invasion and on the levels of VEGF and flt-1 mRNA. Matrigel invasion assay was used to determine the invasion index. Total RNA was isolated from the cells using TRIzol reagent and processed for RT-PCR and results are expressed relative to 18S mRNA.

RESULTS: Our data demonstrates that: 1) IMD protein is expressed at all stages of pregnancy in human placenta; 2) IMD protein is localized in cytotrophoblast cells, syncytiotrophoblast cells and vascular endothelial cells in human placenta; 3) IMD enhances the invasive capacity of first trimester HTR-8SVneo cells by increasing the invasive index to 3.3 compared to 1.2 of the untreated cells; and 4) IMD causes a significant increase (p<0.05) in the levels of VEGF and its receptor, flt-1 in first trimester, HTR-8SV/neo cells.

CONCLUSION: IMD protein is expressed in human placenta at all stages of pregnancy in early human pregnancy. IMD appears to promote angiogenesis during placental development.

456

Human Decidual Macrophages Induce *In Vitro* Capillary-Like Structure Formation in Human Umbilical Vein Endothelial Cells through TNF-α-Mediated ERK1/2 and Ets-1 Activation. Ziming Yu, Dinesh M Shah.* *Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA.*

Background: Placental angiogenesis is fundamental to successful pregnancy and its aberration has been implicated in preeclampsia. Angiogenesis is a tightly regulated process involving an interplay between vascular endothelial cells and various other cell types. It is known that macrophages (MΦs) regulate angiogenesis in wound repair and tumorigenesis. MΦs are the major subset of leukocytes in the human decidua. However, the effect of decidual MΦs on placental angiogenesis has not been established.

Objective: To test our hypothesis that human decidual MΦs induce angiogenesis, at least partially, through TNF-α-mediated ERK1/2 and Ets-1 activation.

Methods: Full-tern normal placentas were obtained within 30 min after vaginal delivery or cesarean section. HUVECs were isolated from the umbilical vein by collagenase infusion and maintained in RPMI-1640 supplemented with 10% FBS and endothelial cell growth supplement. Decidual MΦs were isolated from the decidua vera by enzyme digestion and Percoll centrifugation. The purity of the isolated MΦs was evaluated by the flow cytometric analysis of CD14 expression. To generate cell-free conditioned medium (CM), the isolated MΦs were cultured in FBS-free RPMI-1640 for 48 h and the culture supernatant was harvested, centrifuged and filtered through a 0.22 μM filter. The effect of the decidual MΦ-derived CM on the capillary-like structure (CLS) formation and on the phosphorylation of ERK1/2 and Ets-1 in HUVECs was evaluated, respectively, by *in vitro* angiogenesis assays using Growth Factor Reduced BD Matrigel Matrix and by Western blot analyses. To investigate whether the CM-induced CLS formation and Ets-1 activation was TNF-α- and ERK1/2-dependent, the capacity of an anti-TNF-α antibody and the specific ERK signaling inhibitor, PD98059, to attenuate the induction was determined.

Results: Compared with the FBS-free RPMI-1640, the decidual MΦ-derived CM significantly increased phosphorylation of ERK1/2 at threonine202/tyrosine204 and Ets-1 at threonine38 as well as induced CLS formation in primary HUVECs. Both the neutralizing TNF-α antibody and PD98059 attenuated the CM-induced increase in the phosphorylation of ERK1/2 and Ets-1 as well as the CLS formation.

Conclusions: Human decidual MΦs promote angiogenesis in a paracrine manner, at least partially, through their secretory product TNF-α that induces ERK1/2 and Ets-1 activation.

457

Sera Derived from Normal Pregnant Women and Preeclamptic Patients Influence Endothelial Progenitor Cell Proliferation. Keiichi Matsubara, Emiko Abe, Yuko Matsubara, Masaharu Ito. (SPON: Ronald R Magness). *Obstetrics and Gynecology, Ehime University School of Medicine, Toon, Ehime, Japan.*

Objectives: Preeclampsia is characterized by disturbed neovascularization and hypoxia in the utero-placental circulation. Since endothelial progenitor cells (EPCs) are increased under hypoxic conditions and play an important role in neovascularization, it is possible that EPCs are involved in the pathogenesis of preeclampsia.

Material and Methods: We measured the number of EPCs in nonpregnant women, normal pregnant women, and preeclamptic patients. Peripheral blood was collected from each woman with informed consent. Peripheral blood mononuclear cells (PBMNCs) were isolated using density centrifugation with Ficoll-Paque and incubated with anti- CD133, CD34, and Flk-1 antibody. The number of EPC was measured as CD133+/CD34+/Flk-1+ cells using flowcytometry. Then 8x107 PBMNCs were seeded onto 96-well plates coated with human fibronectin in endothelial basal medium. Adherent cells were cultured through 7 days and analyzed the uptake of acetylated 1,1'–dioctadecyl -3,3,3',3'–tetramethylindocarbocyanine -labeled low density lipoprotein (acLDL-Dil) and FITC-labeled Ulex europaeus agglutinin-1 (FITC-lectin) was analyzed to detect EPCs. Furthermore, PBMNCs were cultivated with serum for 4 days to investigate the effect of serum on EPC proliferation.

Results: The number of EPCs in peripheral blood derived from normal pregnant women did not differ from preeclamptic patients. However, the number of EPCs from preeclamptic patients (2618±642 cells/well) was significantly increased compared with that from normal pregnant women (229±40 cells/well; p<0.001). Serum from preeclamptic patients significantly stimulated EPC proliferation (421±103% vs. control) compared with that from normal pregnant women (142±35% vs. control; p<0.05).

Conclusions: EPCs might be increased and activated by a serum factor related to preeclampsia. It is thought that EPCs are involved in the (maintenance) of the utero-placental circulation in preeclampsia. However, it is thought that serum in preeclampsia contains a factor that inhibits mobilization from bone marrow into peripheral blood.

458

Circulating sFlt-1 Concentration in Preelcamptic Women with Chronic Hypertension or Chronic Proteinuria Does Not Increase during Late Gestation. Bonnie K Dwyer,[1] Sacha Kreig,[1] Raymond R Balise,[2] Jane Chueh,[1] Nihar Nayak,[*1] Maurice Druzin.[*1] *[1]Department of Obstetrics and Gynecology, Stanford University, Stanford, CA, USA; [2]Department of Health Research and Policy, Stanford University, Stanford, CA, USA.*

Objective: Several recent reports suggest an increase in circulating soluble fms-like tyrosine kinase 1 (sFlt-1) and decrease in placental growth factor (PlGF) in pregnancies that subsequently develop preeclampsia (PET). In this prospective controlled study, we examined whether the increased risk of PET in subjects with preexisting medical conditions such as chronic hypertension (cHTN) or chronic proteinuria (cPRO) is associated with an imbalance of circulating pro- and anti-angiogenic factors.

Methods: We enrolled 42 high risk subjects with preexisting medical conditions and 48 normal control subjects. Serum samples were collected at 5 different time periods during pregnancy (<13 weeks, 15-20 weeks, 24-28 weeks, 30-34 weeks and predelivery) for measurement of sFlt-1, PlGF, and vascular endothelial growth factor (VEGF) by ELISA. Angiogenic factors were compared to a primary endpoint of PET and a composite endpoint (PET, small for gestational age, oligohydramnios, or abruption). Two-sided Wilcoxon Rank Sum tests were used for statistical analysis.

Results: 22 subjects developed the composite endpoint (CE), 15 of which had PET. Consistent with earlier findings, after 24-28 weeks of gestation the median sFlt-1 was significantly higher and median PlGF was significantly lower in subjects that developed PET or CE. However, in the subgroups of subjects with cHTN or cPRO who developed CE, the median sFlt-1 level during late gestation (predelivery) was significantly lower than that of the normal control subjects who developed CE (cHTN median 5483 pg/ml vs. control median 15,525 pg/ml, p=0.02; cPRO median 5483 pg/ml vs. control median 15,525 pg/ml, p=0.03).

Conclusion: This is the first prospective study examining the role of angiogenic factors in the development of pre-eclampsia in patients with medical conditions placing them at high risk for PET. Most subjects who developed PET or CE showed elevated sFlt-1 and decreased PlGF as early as 24-28 weeks gestation as expected. However, subjects with cHTN and cPRO who developed CE did not have elevated sFlt-1 predelivery. This result suggests that patients with cHTN or cPRO may have a different mechanism of endothelial cell dysfunction than the controls.

459

The Role of Endothelin-1 in Development of Preeclampsia in Women with Thyroid Disease. Stacy L Strehlow,[1] Martin N Montoro,[1] Frank Stanczyk,[*1] Thomas Goodwin,[*1] Sherfaraz Patel,[2] Jorge H Mestman.[3] *[1]Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, CA, USA; [2]Clinical Labs Group, USC Keck School of Medicine, Los Angeles, CA, USA; [3]Internal Medicine, USC Keck School of Medicine, Los Angeles, CA, USA.*

Objectives:

The objectives of the study were to quantify levels of endothelin-1 in women with hypothyroidism and to establish reference levels of endothelin-1 in pregnancy.

Methods:

Fifteen pregnant women with hypothyroidism and 21 pregnant controls enrolled in the study. Demographic data were collected. Plasma was obtained in the first or second trimester (<27 weeks' gestation) in all subjects and in the third trimester (>34 weeks' gestation) in 13 subjects. Data were also collected on delivery outcomes. Levels of endothelin-1 were determined by QuantiGlo chemiluminescent ELISA.

Results:

For the control population, mean endothelin-1 levels (pg/mL) were 1.81±0.43 in the first trimester, 1.85±0.45 in the second trimester, and 1.95±0.45 in the third trimester. There was no significant difference among the levels in each trimester. The mean endothelin-1 levels in the group of women with hypothyroidism were 1.50±0.55 in the first trimester, 2.02±0.99 in the second trimester, and 1.64±0.30 in the third trimester. Again, no significant difference was found among the levels in each trimester, and the levels were not significantly different from levels in the control population. For the women for whom delivery data were available, 30% (n=10) of the women with hypothyroidism and 15% (n=13) of the controls developed gestational hypertension (chi-square=0.71, NS). Mean endothelin-1 level in women with hypothyroidism who developed gestational hypertension was 1.34 and in controls who developed gestational hypertension 2.04.

Conclusions:

Elevations in endothelin-1 have been associated with development of preeclampsia. Women with hypothyroidism, even when well-controlled, have higher rates of preeclampsia, possibly associated with increased levels of endothelin-1. In this study, levels of endothelin-1 were not elevated in women with hypothyroidism. The rate of preeclampsia was not significantly different, though the number of subjects in the study was limited. Contrary to prior reports, there was no difference in endothelin-1 levels across pregnancy in normal controls in this study.

460

How Does L-Arginine Plus Folic Acid Improve the Reduced Production of Endothelial NO in Rabbit Mesenteric Resistance Artery by Chronic Nitroglycerine Administration? Tamao Yamamoto,[1] Yoshikatsu Suzuki,[1] Yoshimasa Watanabe,[2] Takeo Itoh,[2] Hidetaka Izumi.[*3] *[1]Obstetrics and Gynecology, Nagoya City University, Nagoya, Japan; [2]Cellular Molecular Pharmacology, Nagoya City University, Nagoya, Japan; [3]Obstetrics and Gynecology, Izumi Women Hospital, Fukuoka, Japan.*

We found that reduced action of endothelial nitric oxide (NO) in resistance artery in preeclampsia. We here investigated whether or not the administration of L-arginine plus folic acid improved the reduced action of NO seen in nitroglycerin (NTG) treated rabbit. ACh increased the intracellular NO ($[NO]_i$; estimated using the nitric oxide-sensitive fluorescent dye diaminofluorescein-2) within the endothelial cells of rabbit mesenteric resistance arteries. This was significantly smaller in arteries from NTG-treated rabbits than in those from control rabbits. The reduction in NTG-treated rabbits was prevented when olmesartan (blocker of type 1 angiotensin II receptor (AT_1R)) was co-administered *in vivo* with NTG, and also when the superoxide scavenger manganese (III) tetrakis-(4-benzoic acid) porphyrin (Mn-TBAP), or L-arginine plus the active form of folate (5-methyltetrahydrofolate)) was incubated with the arteries *in vitro*. Endothelial superoxide production (estimated by ethidium fluorescence) was greatly increased in arteries from NTG-treated rabbits. This was normalized by *in vivo* co-administration of olmesartan with NTG and also by *in vitro* application of Mn-TBAP (but not of 5-methyltetrahydrofolate+L-arginine). ACh increased the intracellular Ca^{2+} concentration (estimated using the Ca^{2+}-sensitive dye Fura 2) within endothelial cells, the increase being not significantly different between NTG-treated rabbits and control rabbits.

In conclusion, L-arginine plus folic acid might restore the reduced endothelial NO production due to increasing the bioavailability of L-arginine damaged by superoxide and/or decreasing superoxide production itself in NTG-treated rabbits.

461

Soluble Factors Released from Hypoxic Placental Explants Induce Changes in Endothelial Function Analagous to That of Plasma from Women with Preeclampsia. Nicola J Robinson,[1] Jenny E Myers,[1,2] Richard Blankley,[1,2] Philip N Baker,[*1] John D Aplin,[1] Ian P Crocker.[1] *[1]Maternal & Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom; [2]Michael Barber Centre Mass Spectrometry, University of Macnhester, Manchester, United Kingdom.*

Objectives: Maternal endothelial activation in preeclampsia (PE) is attributed to the release of unknown factors from a hypoperfused placenta. To further characterise these factors we studied the effect of plasma from women with PE in an endothelial bioassay. Factors liberated from serum-free placental villous explant cultures were also investigated in the bioassay.

Methods: Human uterine microvascular endothelial cells were incubated with pooled (control or PE; n=23) plasma samples (10%) before or after 2 types of affinity depletion (removal of 6 or 12 abundant proteins). Cellular ATP levels were measured following 6h exposure using a bioluminescence assay. Term villous explants were cultured in serum-free conditions and exposed to differing oxygen concentrations (20, 6, 1%) to mimic physiological and non-physiological intervillous O_2 tensions. The resulting explant media from day 4 was applied to primary endothelial cells. Conditions were tested in triplicate.

Results: Cells exposed to PE plasma showed reduced cellular ATP levels compared to control plasma (778 ± 51 vs 698 ± 60 RLU). After depletion of abundant proteins, PE plasma showed a greater reduction in ATP when

FRIDAY

compared to depleted plasma from controls (742 ± 25 vs 511 ± 63; 6 proteins) and 667 ± 29 vs 480 ± 134; 12 proteins). As the O_2 concentration of the placental explant medium decreased, a step-wise reduction in ATP was seen ($p<0.0001$, Friedman, n=8). The effect of hypoxic explant media on ATP levels was still evident after removal of particulate material using a 0.22µm filter (885 ± 71 vs 800 ± 20) and following immunodepletion of fibinogen (752 ± 71 vs 587 ± 64).

Conclusions: In endothelial cell cultures, plasma from women with PE reduces cellular ATP. This reduction was maintained following depletion of highly abundant proteins. In a serum-free explant culture model, hypoxia appeared to stimulate release of placental factors which caused a similar reduction in ATP levels. This demonstrates the suitability of the explant model for the identification of soluble factors released from the placenta. We aim to use these parallel strategies in combination with fractionation, depletion and mass spectrometric techniques to identify these factors.

462

Role of Mature and Progenitor Endothelial Cells in Pre-Eclampsia. V Cozzi,[1] G Alvino,[1] S Calabrese,[1] N Fracchiolla,[3] A Martinelli,[1] MC Pasquini,[2] M Cortiana,[3] I Silvestris,[2] A Cortelezzi,[2] Irene Cetin.[*1] *[1]IRCCS F Policlinico, Mangiagalli, Regina Elena, Inst Obs Gyn, Milan, Italy; [2]IRCCS, UO Hem-Trans Dept Med Sciences, Milan, Italy; [3]IRCCS, UO Hem-Trans, Milan, Italy.*

Hypothesis: Endothelial dysfunction of preeclampsia (PE) is part of an excessive maternal response to pregnancy. The manifestations of PE suggest systemic maternal endothelial damage. PE expression on the fetal side is placental insufficiency. Angiogenesis plays a crucial role at the feto-maternal interface. To test the hypothesis that endothelial cells, both mature (CECs) and progenitor (CEPs), may be involved in the pathogenesis of PE, we quantified CEPs and CECs in PE patients compared with normal (N) pregnancies.

Methods: We studied 13 N pregnancies and 17 PE pregnancies in the III trimester matched for gestational age. Maternal age was similar for the two groups, but BMI was significantly higher in the PE vs the control group ($p<0.01$). PE pregnancies delivered earlier and their fetal-placental weights were significantly lower than N pregnancies ($p<0.001$). Total CECs and CEPs were quantified by flow cytometry.

Results: In N pregnancies (n=13) mean values of CECs and CEPs were 20.800/ml (range 9.22-77.114), and 1.051/ml (range 0.067-3.429), respectively. In the PE panel analyzed, represented by 17 cases and 27 samples, the mean values of CECs and CEPs were 25.465/ml (range 1.655-126.344), and 0.418/ml (range 0-15.447). PE cases showed significantly lower CEP than N ($p <0.05$). Dividing the PE group for severity, we compared severe (16 samples), vs mild (11 samples) PE: the severe PE group displayed significantly more CECs ($p <0.02$,), but not CEPs, than controls with mean values of CECs and CEPs that were 29.028/ml (range 1.655-120.780), and 0.512/ml (range 0-15.448) vs 13.578/ml (range 7.667-126.344), and 0.235/ml (range 0-1.410), respectively.

Conclusions: PE is characterized by a significant reduction in CEPs. Considering their potential role in vasculogenesis of the placental bed, this finding is consistent with PE vascular damage leading to impaired fetal growth. CECs, a marker of ongoing endothelial damage, are increased in severe vs mild PE, confirming that PE is characterized by a systemic maternal vascular dysfunction associated with a reduced repairing potential. The increased CECs in severe PE might represent a biological marker that correlates with the severity of the disease.

463

Plasma Asymmetric Dimethylarginine and Circulating Endothelial Progenitor Cells Are Inversely Related in Women with Preeclampsia. Robert W Powers,[*1,2] Patrizia Luppi,[3] Nina Markovic,[2,4] James M Roberts,[*1,2,4] Carl A Hubel.[*1,2] *[1]OB/GYN and Reprod. Sciences; [2]Magee-Womens Research Institute; [3]Pediatrics, Univ. Pittsburgh School of Medicine; [4]Epidemiology, Univ. Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

Bone marrow-derived endothelial progenitor cells (EPCs) enter the systemic circulation to replace defective or injured mature endothelial cells. Nitric oxide is essential for the mobilization and function of EPCs. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS) that suppresses EPC activities in vitro and correlates inversely with EPCs in coronary disease patients. **Objective:** To investigate the relationship between maternal ADMA and EPCs in normal pregnancy and preeclampsia. **Methods:** Primigravid non-smokers with normal pregnancy (control, n=29) or preeclampsia (gestational hypertension, proteinuria and hyperuricemia, n=15) were studied during the 3rd trimester. Patients with multiple gestations, chronic hypertension or other metabolic disorders, or acute inflammatory conditions were excluded. Groups did not differ by gestational weeks at time of venipuncture (means=35) or blood pressure before 20 weeks. EPCs were enumerated by flow cytometry as CD34/kinase insert domain receptor (KDR) and CD133/KDR doubly-positive cells (as percent of total lymphocytes). Data are presented as the percentage of patients with detectable EPCs. Plasma ADMA, arginine (NOS substrate), and biologically inactive symmetric dimethylarginine (SDMA) were measured by HPLC. **Results:**

Variable	Control (n=29)	Preeclampsia (n=15)
ADMA (µM)	0.44±0.01	0.53±0.03*
SDMA (µM)	0.42±0.01	0.54±0.05*
Arginine (µM)	28.3±1.8	29.8±2.2
CD34/KDR positive	22 of 29 (76%)	7 of 15 (47%)*
CD133/KDR positive	23 of 29 (79%)	9 of 15 (60%)*

Mean±SE; *$p<0.05$ vs. control.

Linear regression analysis showed an inverse relationship between maternal plasma ADMA and circulating EPCs; for each 0.05µM increase in ADMA there was an 11% decrease in the number of CD133 positive EPCs ($p<0.05$) and a 15% decrease in CD34 positive EPCs ($p<0.001$). In contrast, there was no relationship between maternal plasma ADMA and KDR positive cells that were negative for CD133 and CD34 (negative control). **Conclusion:** ADMA is higher in women with preeclampsia and is associated with lower circulating EPCs. Funded in part by the PA Dept. of Health and NIH R21HD49453, MO1RR00056 and PO1HD30367.

464

Placental Endoglin Levels Are Increased in Preeclampsia but Not in Pregnancies Complicated by Intrauterine Growth Restriction. Arundhathi Jeyabalan,[*1] Stacy McGonigal,[1] Gail Harger,[2] Ashi Daftary,[1] Augustine Rajakumar.[*1] *[1]Obstetrics, Gynecology and Reproductive Medicine, Magee-Womens Research Institute and University of Pittsburgh, Pittsburgh, PA; [2]Epidemiology, University of Pittsburgh, Pittsburgh, PA.*

Objective: Inadequate trophoblast invasion and spiral artery remodeling leading to poor placental perfusion are believed to underlie preeclampsia (PE) and intrauterine growth restriction (IUGR). Recent studies implicate increased circulating endoglin as a contributor to the pathogenesis of PE along with soluble Flt. We have previously demonstrated that placental hypoxia inducible factor (HIF) and sFlt-1 proteins are upregulated in PE but not IUGR. The objective of this study was to determine whether placental endoglin is altered in pregnancies complicated by PE or IUGR.

Methods: We utilized 10 placentas each from normal pregnant (NP), PE, and IUGR subjects. Diagnosis of PE was based on the Working Group Report (2000) and hyperuricemia of 1 standard deviation above normal levels for gestational age. IUGR (without PE) was defined as normotensive women with infant birth weight less than the 10th percentile and asymmetrical growth profile. Additionally, five placentas from pregnancies complicated by *both* PE and IUGR were evaluated. Endoglin was measured by Western analysis and normalized to β-actin. Densitometric units are presented as mean ± standard error and compared using Kruskal-Wallis and posthoc statistical analysis.

Results: Significant differences in endoglin were observed between NP, PE, and IUGR ($p = 0.02$). Endoglin levels were 2.5-fold higher in preeclamptic placentas compared to NP (14.3 ± 2.8 versus 5.7 ± 1.0, $p < 0.01$). In contrast, endoglin levels were similar in NP and IUGR placentas (5.9 ±1.2 vs 5.7 ± 1.0, p = NS). Placentas from pregnancies with *both* PE and IUGR exhibited marked variability in endoglin levels (mean 12.8, range 2.0 to 37.8).

Conclusions: In contrast to PE, placental endoglin is not increased in placentas from normotensive women delivering small, asymmetrically grown infants. The present study complements our previous findings of elevated HIF and sFlt proteins in PE but not IUGR. The placentas of women with IUGR appear to be fundamentally different from PE women with respect to endoglin, despite the common pathology of deficient trophoblast invasion/spiral artery remodeling and poor placental perfusion.

465

Food Restriction *In Utero* Induces Apoptosis in Offspring Vasculature. Jennifer Halem, Guang Han, Michael Ross,* Omid Khorram. *Obstetrics and Gynecology, Harbor UCLA Medical Center/LA Biomed, Torrance, CA, USA.*

Objective: To test the hypothesis that maternal undernutrition induces cellular apoptosis in offspring vasculature resulting in reduced microvessel density.

Methods: Pregnant Sprague-Dawley rats were fed a standard diet (control) versus a food restriction diet consisting of a 50% decrease in calories from day 10 of gestation to term. Offspring were sacrificed on day 1 of life. Aortas and mesentery from 6 different animals from each dietary group were dissected

and fixed in 4% paraformaldehyde. Five micron paraffin sections of aortas and mesenteric arterioles were made, and subjected to the TUNEL assay. Areas of staining and integrated optical density (IOD) were determined for each vessel using the Image Pro Plus software.

Results: In both the aortas and mesenteric arterioles, a higher percentage of apoptotic cells were found in the food restricted offspring. The area of staining in the food restricted aortas was 73.5 ± 6.59% versus the control group 49.5 ± 3.48% ($P < 0.0001$). The IOD of the food restricted mesenteric arterioles was 38.7 ± 8.9 versus the control group 9.01 ± 4.20 ($P < 0.0001$). Apoptotic cells were noted in both endothelial cells, vascular smooth muscle cells and in the adventitia.

Conclusions: Our results indicate that *in utero* undernutrition induces increased rates of apoptosis in both the conduit and microvessels of the offspring. Increased apoptosis in endothelial cells can lead to rarefaction and may be an etiology of reduced microvessel density and reduced angiogenesis previously reported by us in these offspring. Reduced number of microvessels can lead to increased resistance to flow and contribute to development of hypertension which occurs in adulthood in maternal food restricted offspring.

466

Evaluation of Oxidative Stress Marker (8-oxodG) at Different Gestations in Pregnancy and Its Relationship to Fetal Growth Restriction. Neelam Potdar,[1] John Bankart,[2] Raj Singh,[1] Marcus S Cooke,[1] Justin C Konje.[*1] *[1]Dpt. of Cancer Studies and Molecular Medicine, University of Leicester & University Hospitals of Leicester, Leicester, United Kingdom; [2]Dpt. of Health Sciences, University of Leicester, Leicester, United Kingdom; [3]United Kingdom.*

Introduction

Placental insufficiency is a major source of pro-oxidant reactive species, leading to oxidative stress and cellular damage. In *fetal growth restriction* (FGR), additional to placental ischaemia-reperfusion injury via reactive oxygen species, there is increased oxidative stress in the fetus, leading to higher levels of oxidative stress. We have studied one such marker of oxidative stress, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in the maternal urine.

Objective

To evaluate a biomarker of oxidative stress at different gestations in pregnancy and determine its relationship with fetal growth restriction.

Methods

Prospective case-controlled study of healthy, low-risk pregnant women. Urine samples collected at 12 and 28 weeks of gestation were analysed for DNA derived oxidative stress marker (8-oxodG) by liquid chromatography with tandem mass spectrometry (LC-MS/MS). FGR was defined as birthweight <10th centile based on customised centile charts (www.gestation.net). These constituted the cases and controls were fetuses with normal growth. Statistical analysis was performed using SPSS (Version 12) software.

Results

Two hundred subject samples were analysed for 12 and 28 weeks gestation respectively. The mean values for 8-oxodG for growth restricted and normal pregnancies are given in Table1. As there was a positive skew in the data, it was natural logged for normality. Independent t-test was performed, which showed that the group with normal babies had significantly lower scores than the FGR group (Table 2). The ratio of the geometric means between the two groups were 0.781 at 12 weeks and 0.796 at 28 weeks gestation.

Conclusion

This study shows higher level of oxidative stress marker in pregnancies complicated by FGR as compared to normal outcome pregnancies at different gestations.

Table 1: Log mean values and standard deviation

	8-oxodG log mean values (pmole/µmole creatinine)	Standard deviation
12 week (FGR)	0.849	0.666
12 week (Normal)	0.602	0.751
28 week (FGR)	0.598	0.750
28 week (Normal)	0.370	0.693

Table 2: T -test values for the two groups

	T value	P value	Mean log difference
12 week	-2.47	0.014	-0.24673
28 week	-2.14	0.033	-0.22774

467

Effects of Maternal Vitamin C and E Supplementation on Lipid Peroxide Levels, Total Antioxidant Ability, and Antioxidant Vitamin Levels in Placenta. Yoon Ha Kim,[*1] Tae-Bok Song,[1] Cheol H Kim,[1] Sung Y Yang,[2] Bong W Ahn.[2] *[1]Ob/Gyn, Chonnam National University Medical School, Gwangju, Republic of Korea; [2]Biochemistry, Chonnam National University Medical School, Gwangju, Republic of Korea.*

Objective: Our purpose was to evaluate the effects of maternal vitamin C and E supplementation on lipid peroxide levels, total antioxidant ability, and antioxidant vitamin levels in placental tissues.

Methods: Women at risk for preeclampsia were recruited at 15 to 20 weeks' gestation and randomly assigned to receive either 1000 mg of vitamin C and 400 IU of vitamin E (study group, n=20) or placebo (control group, n=20) daily and take them until delivery. Samples of placental tissue homogenates were obtained after delivery. Lipid peroxide levels were measured by thiobarbituric acid reaction. The oxygen-radical absorbance capacity (ORAC) values were measured by Cao's method. Ascorbic acid, uric acid, β-carotene, retinol, α-tocopherol, and γ-tocopherol were measured by high performance liquid chromatography. Nitric oxide (NO) levels were measured by colormetric nitric oxide assay kit.

Results: 1. Lipid peroxide levels in placental tissue homogenates of the study group were significantly lower than that of the control group (3.59±0.25 vs. 4.31±0.25 nmol/mg protein, $p<0.05$). 2. The ORAC values in placenta tissue homogenates of the study group were significantly higher than that of the control group (9,817.1±222.6 vs. 8,866.4±220.7 U/ml, $p<0.05$). 3. α-tocopherol levels in placental tissue homogenates of the study group were significantly higher than that of the control group (156.8±10.9 vs. 85.8±5.8 nmol/ml, $p<0.01$). 4. There were no significant differences in the levels of ascorbic acid, uric acid, β-carotene, retinol, γ-tocopherol, and NO between each group.

Conclusion: Maternal supplementation with vitamin C and E may be beneficial in the prevention of disease with oxidant-antioxidant imbalance origin in placenta. Multicenter clinical trials with large number of patients will be needed before any decisions regarding clinical practice.

468

Expression of Hemeoxygenase in Human Placental Explants: Effect of Hypoxia-Re-Oxygenation. Fiona Lyall,[*] David McCaig. *Institute of Medical Genetics, University of Glasgow, Glasgow, United Kingdom.*

Introduction: Hemeoxygenase (HO) catalyses the breakdown of heme into iron, biliverdin and carbon monoxide. In many cell types cells studied to date it has been found that the HO-2 isoform is generally constitutively expressed whereas the HO-1 isoform is a stress protein which is rapidly induced in response to a variety of factors. We have previously shown that HO plays an important role in the placenta (1,2,3).

Aim: To determine whether HO plays a role in protecting the placenta from oxidative injury. The first aim was to determine the effect of a fixed period of hypoxia 0%O_2 followed by re-oxygenation, in 5% or 20% O_2, on the expression of HO-1 protein, in villous tissue. The second aim was to determine the effect of cycles of hypoxia followed by re-oxygenation, in 5% or 20% O_2, on the expression of HO-1 protein, in villous tissue.

Methods: 25 villous explants from the same placenta were used for each experiment. For experiment 1 four experimental conditions were set up as well as appropriate controls. These were: A; 1hr h0% O_2, B; 7hr 0% O_2, C; 7 hr 5% or 20% O_2, and D; 1hr 0%O_2 then 6 hr 5% or 20% O_2. For experiment 2, villous explants were cultured in 1, 2 or 3 cycles of 30 min 0% O_2 followed by 30 min 5 or 20% O_2. Again appropriate conrtols were included. HO-1 protein was measured by Western blotting and bands were quantified by densitometry as in previous publications (1,2,3).

Results: There were no statistical differences in HO-1 expression between any of the groups tested for experiment 1 regardless of whether the re-oxygenation was perfomed at 5% or 20% O_2. In experiment 2 exposing the explants to increasing numbers of cycles of hypoxia-reoxygenation still resulted in no significant differences between groups regardless of whether the re-oxygenation was perfomed at 5% or 20% O_2.

Conclusions Under the experimental conditions tested HO-1 expression was not increased in response to hypoxia-re-oxygenation. It remains to be determined whether induction of HO-1 by other, such as chemical, means could protect the placenta against oxidative stress.

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FRIDAY

469

Neutrophils Activate NF-κB in Human Vascular Smooth Muscle Cells. Tanvi J Shah, Sonya Washington, Scott W Walsh.* *OB/GYN, Physiology, Virginia Commonwealth University, Richmond, VA, USA.*

Neutrophil infiltration into systemic blood vessels of preeclamptic women is associated with activation of NF-κB in the vascular smooth muscle. NF-κB is a transcription factor activated by oxidative stress and inflammatory cytokines which regulates the expression of proteins involved in inflammation. Neutrophils release reactive oxygen species (ROS) and TNFα that could be responsible for the activation of NF-κB in vascular tissue. HYPOTHESIS: Activated neutrophils or neutrophil products will activate NF-κB in human vascular smooth muscle cells. METHODS: Primary cultures of human vascular smooth muscle cells (VSMC) were seeded into 24-well plates (40,000 cells/well) and grown to 80% confluence. Cells were transfected with pGL reporter plasmids containing Firefly luciferase for 6 h using Effectene and then treatments were applied overnight. The NF-κB reporter plasmid was constructed from human placental cDNA. A 476 bp segment of the IL-8 promoter containing the NF-κB binding site was isolated, amplified by PCR and cloned into a pGL3 plasmid designated pGL3-BF2. A pGL3-BF2 mutant was constructed by site directed mutagenesis of the NF-κB binding site. Dual luciferase assay was used to measure luminescence after treatments. Neutrophils were isolated by Histopaque density gradient separation and activated with arachidonic acid (AA, 50 μM). RESULTS: Neutrophils treated with AA produced a dose response activation of NF-κB from 1,250 to 10,000 neutrophils/well (80,000 VSMC/well). As compared to 5,000 un-activated neutrophils, 5,000 activated neutrophils caused a 6-fold increase in the activation of NF-κB (44 ± 11 to 256 ± 99 RLU, mean ± SE, n = 7, $P<0.05$). Activation of NF-κB was completely inhibited by co-treatment with SOD/catalase, TNFα neutralizing antibody or by using the mutant plasmid. Treatment with a ROS generating solution (hypoxanthine, 0.05 mM + xanthine oxidase, 0.004 U/mg) stimulated a 4-fold increase in NF-κB ($P<0.05$) which was inhibited by SOD/catalase. Likewise, treatment with TNFα (1 ng/ml) stimulated a 2-fold increase in NF-κB ($P<0.05$) which was inhibited by TNFα neutralizing antibody. CONCLUSION: Activated neutrophils and products released by activated neutrophils (ROS, TNFα) activate NF-κB in human vascular smooth muscle cells. SPECULATION: Infiltration of neutrophils into systemic vascular tissue of preeclamptic women is responsible for the activation of NF-κB and vascular inflammation. HL069851

470

Products of Activated Neutrophils Stimulate Expression of COX-2 and Production of Thromboxane and Interleukin-8 by Human Vascular Smooth Muscle Cells. Tanvi J Shah, Sonya Washington, Scott W Walsh.* *OB/GYN, Physiology, Virginia Commonwealth University, Richmond, VA, USA.*

Neutrophil infiltration of systemic vascular tissue in preeclamptic women is associated with increased expression of cyclooxygenase-2 (COX-2) and interleukin-8 (IL-8) in vascular smooth muscle. COX-2 is the inducible form of cyclooxygenase. Products of COX-2 (prostaglandins and thromboxane) mediate inflammation and vasoconstriction. Activated neutrophils release reactive oxygen species (ROS) and TNFα which could be responsible for increased expression of COX-2 and IL-8. HYPOTHESIS: Products of activated neutrophils will stimulate expression of COX-2 and production of thromboxane (TX) and IL-8 by vascular smooth muscle cells (VSMC). METHODS: Primary cultures of human VSMC were seeded into T-25 flasks and grown to confluence (n = 6). Cells were treated for 18 h with a ROS generating system (hypoxanthine + xanthine oxidase + ferric sulfate); ROS + SOD/catalase; ROS + NS398 (a specific COX-2 inhibitor); TNFα; or TNFα + NS398. RESULTS: Compared to control, treatment of VSMC with ROS caused a greater than 2-fold increase in the expression of COX-2 ($P<0.01$) which was inhibited by co-treatment with SOD/catalase or NS398. Consistent with the increase in COX-2, ROS significantly increased the production of TX by 2-fold (0.8 ± 0.1 to 1.9 ± 0.1 pg/μg protein, $P<0.05$). ROS also stimulated VSMC production of IL-8 (2.4 ± 0.6 to 7.4 ± 1.8 pg/μg protein, $P<0.05$). SOD/catalase and NS398 inhibited the ability of ROS to stimulate TX and IL-8. TNFα stimulated a 3-fold increase in the expression of COX-2 ($P<0.001$). TNFα increased TX (1.7 ± 0.3 to 2.8 ± 0.7 pg/μg protein) and IL-8 (0.3 ± 0.1 to 6.6 ± 2.7 pg/μg protein, $P<0.05$). In all cases the stimulatory effect of TNFα was inhibited by co-treatment with NS398. CONCLUSION: Products released by activated neutrophils (ROS, TNFα) induce expression of COX-2 in vascular smooth muscle cells and stimulate the production of TX and IL-8. SPECULATION: Release of ROS and TNFα by neutrophils that have infiltrated systemic vascular tissue is responsible for increased expression of COX-2 and IL-8 in women with preeclampsia. Linked to the increased expression of COX-2 was a significant increase in the production of TX. TX is a potent vasoconstrictor, so neutrophil infiltration into systemic vascular tissue of preeclamptic women could be responsible for increasing vascular tone leading to hypertension. HL069851

471

Proteomic Profiling of the Placenta Identifies Increased Hsp27 Expression in Preeclampsia. Brad A Pitzer, Diane E Brockman, Victoria H Roberts, Leslie Myatt,* Rose P Webster. *OB/GYN, University of Cincinnati, Cincinnati, OH, USA.*

Introduction: According to current opinion the primary pathology for the disease is placental in origin resulting in altered placental functions. We hypothesized that preeclampsia would involve distinct changes in placental protein expression patterns that could be studied using proteomics.

Objective: Our aim was to investigate differences in placental protein expression patterns in preeclampsia using two-dimensional electrophoresis (2DE) and to validate the differences obtained by other independent methods.

Methods: Villous tissue from normal or preeclamptic placentae was lysed at 4ºC using lysis buffer (2% CHAPS, 20 mM Tris pH 7.5, 1mM EDTA, 1 mM EGTA and protease inhibitors). Following centrifugation at 20,000xg at 4ºC for 5 min, the supernatant was used for 2D electrophoresis (n=3) and western blot (n=6). Gels were analyzed using HT Investigator gel analysis software to identify differences in protein expression. Differentially expressed proteins were then identified by LC-MS. Western blot and immunohistochemistry was carried out with antibodies to Hsp27.

Results: Comparison of protein expression patterns between normal and preeclamptic placenta identified seven spots that differed in intensity. Protein spot number 238 was 4x more intense in preeclampsia as compared to normal and was identified to be Hsp27 on the basis of 5 peptide matches. Western blot data confirmed that placental levels of Hsp27 were significantly greater ($p<0.01$) in preeclampsia. Immunohistochemical analysis localized Hsp27 predominantly in the synctiotrophoblast (ST) and vascular smooth muscle layer of the placenta with a trend towards greater staining intensity in the ST and smooth muscle layer in the preeclamptic group as compared to normotensive. Weak Hsp27 staining was observed in the stroma and the endothelium.

Conclusions: We have here shown using 2DE, LC-MS and western blot that Hsp27 is significantly increased ($p<0.01$) in preeclampsia. Hsp27 belongs to the group of heat shock proteins that are induced by stress and function as molecular chaperones to help cells survive in a stressed environment. We speculate that the heightened oxidative stress environment prevalent in preeclampsia induces an increase in Hsp27. Hsp27, being anti-apoptotic is also induced during apoptosis, which also exists in the preeclamptic placenta.

472

Differential Cytokine Responses of First Trimester Trophoblast Cells to Viral and Bacterial Products. Vikki M Abrahams,[1] Paulomi B Aldo,[1] Irene Visintin,[1] Roberto Romero,*[2] Gil Mor.*[1] *[1]Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA; [2]The Perinatology Research Branch, NICHD/NIH, Detroit, MI, USA.*

Objectives: Infections pose a significant threat to pregnancy. The trophoblast plays an active role in the protection against infection by promoting an immune response and producing anti-microbial factors. One of the mechanisms by which trophoblast cells recognize microbial products is through the expression of Toll-like receptors (TLR). We hypothesize that the trophoblast generates a specific cytokine response depending on the type of microbial stimuli. The objective of this study was to characterize the trophoblast cytokine profile following bacterial and viral stimulation.

Methods: The human first trimester trophoblast cell line, HTR8, was incubated with or without the TLR-4 ligand, bacterial lipopolysaccharide (LPS; 100μg/ml) or the TLR-3 ligand, Poly(I:C) (synthetic viral dsRNA; 25μg/ml). Cytokine expression in cell lysates and secretion into supernatants was quantified using the Beadlyte Multiplex Assay with detection and analysis using the Luminex 100 IS.

Results: Significant differences were observed in the trophoblast cytokine profile following bacterial and viral stimulation. Only high doses of LPS (100μg/ml) induced a moderate increase in the secretion of IL-6 (1.7 fold), MCP-1 (1.4 fold), IL-8 (1.9 fold), GROα (2.1 fold) and this was seen only after 72 hours of treatment. In contrast, the viral stimuli, Poly(I:C), triggered a rapid and robust response, characterized by an increase in both the expression and secretion of cytokines. Increased secretion of IL-6 (28.3 fold), MCP-1 (18.6 fold), IL-8 (25 fold) RANTES (132 fold) and GROα (11.6 fold) was seen after 24 hours of Poly(I:C) treatment.

Conclusions: This present study demonstrates that first trimester trophoblast cells differentially respond to bacterial and viral products by generating diverse intracellular and secreted cytokine profiles at distinct rates. The rapid, robust response to viral products compared with the slower, milder response towards bacterial products suggests that viral infections pose a greater threat to the placenta and pregnancy.

473

Characteization of Cynomolgus and Vervet Monkeys Decidual Lymphocyte Populations: Prevalence of Low Cytotoxic CD56$^+$ Perforin$^+$ Cells within NK Cells and CD8$^+$ Phenotype of T Cells. Svetlana Dambaeva,[1] Mark Garthwaite,[1,2] Maureen Durning,[2] Thaddeus Golos.[1,2] (SPON: Ronald R Magness). *[1]Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA; [2]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, USA.*

The decidua is a specialized adaptation of the uterine endometrium to support the implanting embryo in primate pregnancy. Non-human primates are the important animal models for study of maternal immune response within decidua to fetal development. The objective of this study was the phenotypic and functional evaluation of decidual immune cells in the cynomolgus and vervet (African green) monkeys.

Methods: early pregnancy (d36-42) cynomolgus and vervet deciduas were obtained by cesarean section. Lymphocytes were isolated by enzymatic dispersion and gradient centrifugation. Multi-color flow cytometry was used to phenotype cell populations, chromium release assay was used to evaluate cytotoxic activity of NK cells.

Results: NK cells were found as the richest lymphocyte population in the monkeys deciduas and the majority were CD56 bright cells, like human decidual NK (dNK) cells. Peripheral NK (pNK) cells in cynomolgus and vervet, in contrast to human, do not express CD56, suggesting the influence of a unique decidual environment to promote NK cell differentiation. While human CD56$^+$ dNK cells are CD16$^-$, more then 70% of monkeys' CD56$^+$ dNK cells remained CD16$^+$. Intracellular staining with anti-perforin mAbs demonstrated that monkeys' dNK cells contain this protein. However, the cytotoxic potential was not apparent in chromium assay; CD56$^+$ cells from cynomolgus decidua showed strongly reduced cytolytic activity against target cells when compared to pNK cells. Approximately 10% of decidual leukocytes are T cells. Most decidual T cells in cynomolgus and vervet were phenotyped as cytotoxic CD8$^+$ cells (about 70%) and 2-15% co-expressed the NK cell marker CD56 (NKT cells). The $\gamma\delta$ T cell receptor was identified on 5.5±2.1% in cynomolgus and 3.7±1.6% in vervet of decidual T cells, and two subsets of $\gamma\delta$ T cells could be distinguished: CD3$^+$56$^+$, and 3$^+$56$^-$ cells.

Conclusion: Together, these results showed that cynomolgus and vervet maternal-fetal interfaces are very rich in immune cells. Lymphocytes populations' diversity in monkeys' deciduas correlate with human studies, confirming that both species are excellent models for study mechanisms of immune recognition and tolerance that support primate pregnancy.

474

Trophoblast-Macrophage Interactions: A Regulatory Network for the Protection of Pregnancy. Stefan Fest,[2] Paulomi B Aldo,[1] Vikki M Abrahams,[1] Roberto Romero,[*3] Gil Mor.[*1] *[1]Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Paediatrics, Charité Universitätsmedizin, Berlin, Germany; [3]The Perinatology Research Branch, National Institute of Child Health and Human Development, Detroit, MI, USA.*

Background: Macrophages are one of the first immune cells observed at the implantation site. Their presence has been explained as the result of an immune response towards paternal antigens. The mechanisms regulating monocyte migration and differentiation at the implantation site are largely unknown. In the present study we demonstrate that trophoblast cells regulate monocyte migration and differentiation. We propose that trophoblast cells "educate" monocytes/macrophages to create an adequate environment that promote trophoblast survival.

Methods: CD14+ monocytes were isolated from peripheral blood using magnetic beads. Co-culture experiments were conducted using a two-chamber system. Monocytes were stimulated with lipopolysaccharide (LPS) and cytokine levels were determined using Multiplex cytokine detecting assay.

Results: Trophoblast cells increase monocyte migration and induce a significant increase in the secretion and production of the pro-inflammatory cytokines (IL-6, IL-8, TNF-α) and chemokines (GRO-α, MCP-1, MIP-1β, RANTES). Furthermore, the response of monocytes to LPS was different in monocytes pre-exposed to trophoblast cells.

Conclusions: The results of this study suggest that trophoblast cells are able to recruit and successfully educate monocytes to produce and secrete a pro-inflammatory cytokine and chemokine profile supporting its growth and survival. Furthermore we demonstrate that trophoblast cells can modulate monocytes response to bacterial stimuli.

475

Soluble Triggering Receptors of Myeloid Cell (sTREM-1) Is Increased in the Amniotic Fluid of Women with Spontaneous Preterm Birth. Stepehn J Fortunato, Salvatore J Lombardi, Ramkumar Menon. (SPON: Kelle H Moley). *The Perinatal Research Center, Nashville, TN, USA.*

OBJECTIVE: TREM1 is expressed by neutrophils and macrophages and enhances the innate immune response. TREM1, along with bacterial antigens and their recognition molecules such as toll like receptors, intensify the proinflammatory response by releasing an array of cytokines and other inflammatory mediators. The presence of soluble forms of TREM-1(sTREM1) is evidence of an overwhelming inflammatory response. The presence of sTREM1 has not been studied in preterm birth (PTB) where inflammatory mediators play a major role triggering early onset of labor. This study examines the presence or absence of TREM1 in the amniotic fluid (AF) of women with preterm birth (PTB). Since race is a risk modifier in PTB we examined the data stratified by race to determine any ethnic disparity in sTREM concentrations between African-Americans (AA) and Caucasians (C).

METHODS: In this case (PTB ≤ 36 weeks gestation) control (normal term delivery > 37 weeks) study 198 AF samples were collected (147 cases [39 AA and 62 C] and 174 controls [48 AA and 51 C]) at the time of active labor. Microbial invasion of the intraamniotic cavity (MIAC) was documented in the AF detected by amplification of microbial 16s ribosomal DNA by polymerase chain reaction. sTREM concentration was measured by ELISA. Median differences (pg/ml) between cases and controls were examined by Wilcoxon Ranked test.

RESULTS: sTREM1 was measurable in all AF samples and in a pooled analysis (AA+C) the median concentration of sTREM1 was significantly higher in cases (1176) than in controls (716; $p < 0.0001$). In cases, MIAC was associated with a significant increase in sTREM1 (median 2762) compared to cases with no MIAC (1182; $p = 0.008$). When data was stratified by race, a significant increase in sTREM was seen in cases from both races. MIAC was associated with significantly higher sTREM in both races compared to non MIAC cases.

CONCLUSIONS: Herein we document the presence of a novel family of proinflammatory mediators in the AF of women during pregnancy. As an enhancer of inflammation sTREM elevation in the AF at the time of PTL and even higher concentrations in cases with MIAC suggests its potential role in modulating the immune response and triggering PTB. The exact role of sTREM in the AF is not clear, however, as an activator of cytokine release, sTREM increase indicates a role in inflammation induced PTB independent of the ethnic variation.

476

Evidence for Phenotypic Versatility of Chorioamniotic Mesodermal Cells and a Potential Role for an ETS Family Transcription Factor PU.1. Chong Jai Kim,[1,2] Roberto Romero,[*1,3] Sung-Su Kim,[1] Asad Abbas,[1] Jung-Sun Kim,[1] Yeon Mee Kim,[1,2] Enola Cushenberry,[1] Jimmy Espinoza.[1,4] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Pathology, Wayne State University, Detroit, MI, USA; [3]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Objective: Fetal membranes play a central role in pregnancy and parturition. While amnion epithelial cells and trophoblasts have been characterized, the nature of mesodermal cells within the amnion and chorion remains unclear. The controversy lies in whether these cells represent fibroblasts, myofibroblasts, and/or macrophages. This study was performed to determine whether chorioamniotic mesodermal cells switch phenotypes.

Methods: Fetal membranes were analyzed by immunohistochemistry and immunofluorescence using a panel of antibodies to CD14, CD163, α-smooth muscle actin (αSMA), desmin, vimentin, and PU.1. Membranes studied included the zone of altered morphology (ZAM), meconium stained membranes, and acute chorioamnionitis. The proportion of CD163+ macrophages was compared between membranes from cases with preterm delivery (PTD) with (n=12) and without (n=10) chorioamnionitis. Chorionic mesenchymal cells were transfected with PU.1 cDNA and changes in CD14 mRNA expression and CD14+ population were evaluated by qRT-PCR and flow cytometry.

FRIDAY

Results: 1) Three distinct phenotypes- fibroblasts, myofibroblasts, and macrophages- were demonstrated among mesenchymal cells; 2) CD14+/ αSMA+ cells (macrophage-myofibroblast phenotype) were frequently encountered, especially in the ZAM; 3) The cells expressing CD14 and CD163 were PU.1+; 4) The percentage of CD163+ cells was higher in PTD with chorioamnionitis than in PTD without ($p<0.05$); 5) Transfection with PU.1 cDNA increased the CD14 mRNA expression and the proportion of CD14+ cells.
Conclusion: 1) We report for the first time that chorioamniotic mesodermal cells are phenotypically versatile. Such changes encompass transformation of fibroblasts to overt macrophages; 2) Alterations in the proportion of fibroblasts, myofibroblasts, and macrophages perhaps reflect biological events required for the maintenance of membrane integrity, response to stretching, membrane rupture, and immunity; 3) Elucidation of signals responsible for phenotype versatility is crucial for understanding the biology of human pregnancy.

477

Controlled Ovarian Stimulation as Treatment in Unexplained Recurrent Miscarriage. Federico Mecacci,[1] Ilaria Vicini,[1] Carolina Becattini,[1] Riccardo Cioni,[1] Carlotta Buzzoni,[1] Elisabetta Coccia,[1] M Rosaria Di Tommaso,[1] Michael Paidas.[2] (SPON: Charles J Lockwood). *[1]Obstetrics and Gynecology, Careggi University, Florence, Italy; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Background: Approximately 50% of recurrent miscarriage remains unexplained, without an effective treatment.
Objective: To test the hypothesis that controlled ovarian stimulation by gonadotropins enhances the viable pregnancy rate in women with recurrent miscarriage
Methods: In this retrospective, observational study, the study group consisted of 62 subjects with unexplained recurrent miscarriage treated with recombinant Follicle Stimulating Hormone (rFSH), human chorionic gonadotropin and micronized vaginal progesterone, while a historical control group of 30 women with unexplained recurrent miscarriage were treated with only micronized vaginal progesterone.
Results: In the study group, the total pregnancy rate was 77.4%. After controlled ovarian stimulation, the viable pregnancy rate was 83.3% and miscarriage rate was 16.7%, significantly lower ($p<0.05$) than that of historical group (36.6%).
Conclusions: Our findings suggest that controlled ovarian stimulation with rFSH could be an attractive treatment for unexplained recurrent abortion.

478

Response of Placental Explants from Term and Preterm Pregnancies to an Immune Challenge. Naomi M Scott,[1] Michael J Stark,[1] Ian MR Wright,[1] Deborah M Hodgson,[1,2] Roger Smith,[*1] Vicki L Clifton.[*1] *[1]Dept of Endocrinology, Mothers and Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia; [2]Laboratory of Neuroimmunology, University of Newcastle, Newcastle, NSW, Australia.*
Background: The regulation of cortisol availability by the placenta plays an important role in a successful pregnancy. We have shown cortisol bioavailability and regulation differ with gestation, fetal sex and the presence of a number of complications during pregnancy including preterm birth and asthma. Alterations in cortisol concentrations may alter placental immune pathways particularly the balance between Th1 and Th2 cytokines. We examined the placental response to an immune challenge in preterm and term infants in the presence or absence of glucocorticoids, with respect to neonatal sex.
Methods: Placentae were collected from normal term deliveries and preterm deliveries of pregnancies complicated by pre-eclampsia and IUGR. Placental explants were cultured for 24hrs and then exposed to lipopolysaccharide (LPS) (1ng/ml), in the presence and absence of 100nM dexamethasone, 1mM cortisol or 10mM cortisone. After 24hrs the supernatants were collected, and assayed for TNF alpha by sandwich ELISA.
Results: Placentae from term deliveries had a significantly higher TNF alpha response to LPS than preterm placentae ($p<0.05$). The TNF alpha response to LPS was significantly inhibited by dexamethasone and cortisol in both term and preterm placentae. The percentage inhibition of the TNF alpha production by dexamethasone and cortisol was significantly greater at term when compared to preterm placentae. No significant differences in response to LPS were observed in placentae from male and female fetuses. However, 50% of placentae from the preterm male neonates demonstrated a minimal response to LPS with no inhibition of placental TNF alpha production by glucocorticoids.
Conclusion: This data supports that the normal pro-inflammatory response to an immune challenge is a function of gestational age. An increase in glucocorticoid sensitivity with advancing gestation is supported by the greater immuno-modulatory effects of glucocorticoids observed at term. A lower response to an immune challenge and relative glucocorticoid resistance was observed in preterm males. This may represent a sexually dimorphic change in glucocorticoid metabolism in response to a maternal inflammatory stressor and contribute to the observed male excess in morbidity and mortality following preterm birth.

479

Measurement of Inflammation-Stimulated Prostaglandins from Human Gestational Membranes. Natalie W Thiex, Sarah R Thiel, Rita Loch Caruso. (SPON: Timothy RB Johnson). *Environmental Health Sciences, Toxicology Program, University of Michigan, Ann Arbor, MI, USA.*
Objective: Determine prostaglandin (PG) availability to the myometrium after inflammatory stimulus by measuring discrete release from amnion or choriodecidua after treatment by tumor necrosis factor α (TNFα), lipopolysaccharide (LPS) or environmental toxicants.
Methods: Gestational membranes were collected from healthy term (37-39 weeks gestation) human pregnancies via scheduled Cesarean section deliveries. Sections of gestational membranes with intact amnion and choriodecidua were mounted onto 12mm Transwell™ frames to create distinct amnion and choriodecidual chambers. The tissue was incubated under normal conditions and treated with TNFα, LPS, or environmental toxicants for 1-24 hours. Using this system, PGs and cytokines secreted into the culture supernatant were measured discretely from the amnion and choriodecidual chambers by enzyme immunoassays. After the culture supernatant was collected, some tissue was removed from the Transwell™ frames, snap frozen and processed for immunoblot analysis to determine tissue protein concentrations (PGHS2, PGDH & PGT). The remaining tissue was processed for immunohistochemistry: Slides containing 6-8μm frozen sections were analyzed for presence of proteins related to cytokine and PG metabolism and fuction and cellular markers. The antigens were labelled with a biotinylated secondary antibody and an avidin: biotinylated enzyme complex, visualized with 3,3'-diaminobenzidine and counterstained with hematoxylin. Stained sections were visualized with an upright light microscope and recorded digitally.
Results: TNFα and LPS increased the production of PGE_2 and $PGF_{2\alpha}$ in the supernatant collected from the amnion side of the membranes, but not in the supernatant from the choriodecidual side.
Conclusions: Previous research conducted on explanted tissue without distinction of amniotic or choriodecidual contributions or isolated cells from gestational membranes showed an increase in uterotonic PGs upon inflammatory stimulus. Historically results such as these have been interpreted to mean that those PGs acted on the myometrium to cause uterine contraction. However, my work indicates that there is no change in PG release on the choriodecidual side of the membrane suggesting that inflammation-stimulated bioactive PGs do not access the myometrium to effect uterine contractions. It remains possible that amniotic PGs affect uterine contraction or parturition via other pathways.

480

Dominant TGFβ1, TGFβ-R1, CD105, and CD133 Expression in Trophoblasts of the First Trimester Human Placenta Implicates Immunoregulatory and Angiogenic Potential. Jingxia Sun,[1] Yanping Zhang,[2] Xin Gu,[3] David F Lewis,[2] Yuping Wang.[*2] *[1]Obstetrics and Gynecology, First Hospital, Harbin Medical University, Harbin, Heilongjiang, China; [2]Obstetrics and Gynecology; [3]Pathology, LSUHSC-Shreveport, Shreveport, LA, USA.*
Objective: Transforming growth factor-β (TGFβ) superfamily members play an integral role during placental development. CD105 and CD133 are stem cell markers for embryo tissue. The purpose of this study was to examine the expression and localization for TGFβ1, TGFβ receptor1 (TGFβ-R1), CD105 and CD133 in the first trimester placenta. Placentas from early third trimester and at term were used as comparisons.
Methods: First trimester human placentas (n=6) were obtained by curettage during therapeutic termination at 6-10 weeks of gestation. Early third trimester (32-34 weeks, n=4) and term (37-40 weeks, n=4) placentas were obtained from normal pregnant women after selective c/s or vaginal delivery. Placental tissue pieces were fixed with formalin and embedded in paraffin. TGFβ1, TGFβ-R1, CD105 and CD133 immunoactivities were examined by immunohistochemical staining of villous tissue sections. Sections stained without primary antibodies were used as the negative control.

Results: TGFβ1 and TGFβ-R1 were abundantly expressed in cytotrophoblasts (CT) and syncytio-trophoblasts (ST) of the first trimester placentas, especially in villous column formation sites. TGFβ1 and TGFβ-R1 were less expressed in ST of early third trimester placentas than those in the first trimester and weakly expressed in ST of term placentas. Immuno-reactivity for CD105 and CD133 were also localized and intensively expressed in both CT and ST of the first trimester placentas. CD105 expression was much reduced in ST in the early third and almost lost in the term normal placentas. The pattern of CD133 immunoreactivity was similar to that of CD105.
Conclusions: The intensive imunoreactivities of TGFβ1, TGFβ-R1, CD105 and CD133 in CT and ST of the first trimester placenta implicate that these growth factors play an important role in modulating villous out growth. Since CD105 and CD133 are involved in angiogenesis and cell proliferation and differentiation processes, the differential expression patterns of CD105 and CD133 from TGFβ1 and TGFβ-R1 suggest the possible autocrinal and paracrinal regulations of immunoregulatory and angiogenic reactions among these factors during the early placental development and diverse regulatory pathways in the late pregnancy.

481

A Sequence in the 5' Flanking Region on the Human Bcl-2 Gene Confers Progestin Responsiveness in Uterine Leiomyoma Cells In Vitro. Ping Yin,[1] Zhihong Lin,[1] Youhong Cheng,[1] Erica E Marsh,[1,2] Hiroki Utsunomiya,[1] Hiroshi Ishikawa,[1] Scott Reierstad,[1] Julie Kim,[*1] Eugene Xu,[1] Serdar Bulun.[*1,2] *[1]Division of Reproductive Biology, Northwestern University, Chicago, IL, USA; [2]Reproductive Endocrinology and Infertility Division, Department of Obstetrics and Gynecology, Feinberg School of Medicine-Northwestern University, Chicago, IL, USA.*
Objective: Progesterone antagonists were shown to reduce the size of leiomyomata and improve symptoms. Previous studies showed that progestins stimulate the proliferation of the human uterine leiomyoma cells in culture. We and others previously reported that mRNA and protein levels of Bcl-2, an anti-apoptotic gene, are stimulated by progestins in uterine leiomyoma cells. In this study, we explore the hypothesis that progestins regulate the Bcl-2 gene promoter.
Methods and Results: We employed primary cultures of human leiomyoma smooth muscle cells from 10 patients as a model system. Using the Transcription Element Search System (TESS, http://www.cbil.upenn.edu/tess/), a 15 bp sequence GGATCAtgcTGTACT at -534/-520 from the ATG start site was identified in the Bcl-2 promoter, which is highly homologous to the progesterone response element (PRE) consensus sequence. Gel shift assay indicated that this sequence interacted with the nuclear extracts from leiomyoma cells treated with progesterone for 2 hours. This interaction was specific as shown by a cold competitor probe, and a supershift showed the presence of PR in the DNA-protein complex. To investigate whether Bcl-2 promoter activity is regulated by progestins, we cloned the -631/+89 fragment of this promoter containing the putative PRE into a luciferase reporter vector and showed that the Bcl-2 promoter activity was upregulated by R5020, a progesterone agonist, in leiomyoma cells cotransfected with plasmids expressing the full length PR isoform PR-B, but not with those expressing the truncated PR isoform PR-A or empty vector. Finally, we observed by chromatin immunoprecipitation (ChIP) analysis that PR is recruited to the Bcl-2 promoter in leiomyoma cells stimulated with progesterone.
Conclusions: we demonstrated for the first time that the Bcl-2 promoter contains a functional PRE that binds PR and that its activity is upregulated by progesterone or its analogs in primary uterine leiomyoma cells. Our results suggest that ligand-binded PR regulates Bcl-2 transcription via directly binding to its promoter.
Supported by the NIH grant HD46260.

482

The Expression Profiling of Micro-RNA in Leiomyomas, Tumor Progression and Regulation by Ovarian Steroids and TGF-ß. Qun Pan, Xiaoping Luo, Nasser Chegini.[*] *OB/GYN, University of Florida, Gainesville, FL, USA.*
MicroRNAs (miRNAs) are a newly identified class of non-protein-coding small RNAs of approximately 20nt with critical function in multiple biological processes through mRNAs degradation or repression. Since alteration in miRNA expression is considered to contribute to cellular machinery that regulates various cellular activities implicated in normal and disease processes, including cancer we profiled their expression in myometrium and leiomyoma. Using miRNA microarray we captured the expression profile of 207miRNA in normal myometrium and matched leiomyomas, their isolated smooth muscle cells (MSMC,LSMC), in a LSMC culture spontaneously transformed after third passage and in SKLM, a leiomyosarcoma cell line. The results indicated that myometrium express the highest number of miRNA (N=80) as compared to leiomyomas with further decline in expression in MSMC and LSMC derived from the same tissues. We also observed a decline in the number of miRNA expression in the transformed LSMC as compared to MSMC and LSMC, with lowest number of miRNA expression in SKLM as compared to other cell types. We confirmed the expression of three of the miRNA, mir20, mir21 and mir26a in these tissues and cells using realtime PCR. In addition, we demonstrated that the expression of these miRNA is regulated by estrogen, progesterone and TGF-ß. The results provide the first example of miRNA profile in myometrium and leiomyomas and during cellular transformation from normal to sarcoma. Because of a considerable decline in the expression of a number of these miRNA our results suggest that aberrant expression of these selective miRNA are involved in the possible development of leiomyoma and their progression from benign into cancerous stages. (Supported by grant HD37432).

483

Peroxynitrite Plays a Critical Role in Caspase-3 Mediated Apoptosis of Normal Peritoneal Fibroblasts. Ghassan M Saed, Zhong L Jiang, Michael P Diamond,[*] Husam M Abu-Soud. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*
Introduction: Adhesion fibroblasts are characterized by low levels of nitric oxide (NO), lower apoptosis, and higher protein nitration than normal fibroblasts. The hypothesis to be tested is that the lower levels of NO observed in adhesion fibroblasts are due to the increased consumption of NO by superoxide to form peroxinitrite, a powerful nitrating agent in a biological system. Peroxinitrite is used for caspase-3 nitration, which leads to the loss of its activity, and subsequently, decreases apoptosis in adhesion fibroblasts.
Objective: To test the effects of peroxinitrite in the presence and absence of superoxide dismutase (SOD), a superoxide scavenger, on caspase-3 mediated apoptosis in normal and adhesion fibroblasts under normal and hypoxic conditions.
Methods: Fibroblasts from normal peritoneal and adhesion tissues were cultured under normal (20% O_2) and hypoxic (2%) conditions and exposed to increasing concentrations of peroxinitrite (0.5, 1.0, 1.5 μM) for 20 min with and without (SOD, 10 U/ml) treatment. Caspase-3 activity and apoptosis were measured by colormetric and TUNEL assays, respectively.
Results: Caspase-3 activity was 30% lower in adhesion as compared to normal fibroblasts. Hypoxia treatment resulted in a significant decrease in caspase-3 activity and apoptosis in normal fibroblast with little or no change in adhesion fibroblasts. Treatment of normal and adhesion fibroblasts with peroxinitrite resulted in a significant decrease in caspase-3 activity in both fibroblasts under normal and hypoxic conditions. Treatment with SOD partially prevented the loss in caspase-3 activity at 0.5 μM peroxinitrite concentration, but, it failed to prevent it at 1.0 μM and higher peroxinitrite concentrations in normal and adhesion fibroblasts.
Conclusions: Our data suggests that the reduced levels of NO observed in adhesion fibroblasts are due to the presence of higher levels of superoxide which combine with NO to form peroxinitrite. Nitration of caspase-3 lead to a significant decrease in its activity and resulted in lower apoptosis. Removal of superoxide by SOD resulted in a partial restoration of caspase-3 activity and increase in apoptosis. This may be a potential therapeutic intervention for the elimination of the adhesion fibroblast phenotype during peritoneal healing.

484

Endothelin System Expression in Ovarian Follicles of Women with Polycystic Ovary Syndrome. Tal Imbar,[1] Eyal Klipper,[2] Ronit Haimov-Kochman,[*1] Arye Hurwitz,[1] Caryn Greenfield,[1] Rina Meidan.[2] *[1]Obstetric & Gynecology, Hadassah Mt. Scopus, The Hebrew University Medical Center, Jerusalem, Israel; [2]Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University, Rehovot, Israel.*
Objective: Elevated plasma Endothelin-1(ET-1) has been reported in some insulin resistant patients and also in Poly Cystic Ovaries Syndrome (PCOS) patients. We examined the levels of ET-1 system in granulose lutein cells of PCOS patients in comparison to normally ovulating women undergoing In-Vitro Fertilization (IVF).
Materials & methods:Patients with PCOS and normal ovulating women (control group) reaching IVF underwent the long suppression protocol, utilizing gonadotropin-releasing hormone agonist. Follicular aspirates containing granulosa lutein cells (GLC) were obtained from PCOS and control during

FRIDAY

oocyte retrieval. RNA and proteins were extracted and endothelin converting enzyme-1 (ECE-1), ET-1 and type A endothelin receptors (ETA) were quantified using real time PCR and western blot analysis.
Results: Seven PCOS patients complying with the Rotterdam consensus diagnostic criteria and their matched controls were analyzed in this study. Real-time PCR analysis revealed that that preproET-1 in PCOS patients were higher than in control group (6.5 ± 1.7 vs. 1.4 ± 0.42). mRNA expression of endothelin converting enzyme –1 (ECE-1) responsible for producing active ET-1, was also elevated in GLC of PCOS patients. The presence of ETA in GLC, in both groups of patients, supports an autocrine action of ET-1 within the follicle.
Conclusions: Enhanced expression of ECE-1 and ET-1 mRNA in the granulosa cell layer of women with PCOS may be a diagnostic and pathological marker of this condition associated with low fertility. Elevated ET-1 levels may interfere with the ovulation process and luteal development.

485

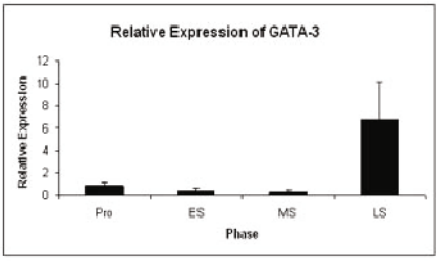
Estrogen Replacement Enhances EDHF-Mediated Vasodilation of Rat Uterine Radial Arteries. Natalie Z Burger, George Osol,* Natalia I Gokina. *Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*
Introduction: Our previous data indicate that EDHF-mediated uterine vasodilation is significantly potentiated in rat pregnancy. We hypothesized that estrogen might be an important factor modulating EDHF-mediated vasodilation of uterine radial arteries. The objectives of the present study were: (1) to characterize acetylcholine (ACh)-induced nitric oxide (NO)- and prostacyclin (PGI_2)-independent vasodilation of uterine arteries from estrogen-deficient (ovariectomized) rats; (2) to determine the effect of estrogen replacement on EDHF-mediated uterine vasodilation.
Methods: Radial uterine arteries were dissected from the uteri of ovariectomized untreated (OVX) and 17ß-estradiol-replaced (OVX+E) rats. The arteries were cannulated and pressurized to 50 mmHg. The production of NO and PGI_2 was inhibited with LNNA (200 μM) and indomethacin (10 μM), respectively. The vessels were pre-constricted with phenylephrine to 50-70% of the initial diameter, and ACh was added in increasing concentrations in 5-minute intervals. A combination of papaverine and diltiazem was used at the end of each experiment to obtain maximally-dilated arterial diameters (D_{max}).The transient and sustained changes in the arterial diameter were recorded. Concentration-response curves were obtained for each group, and the concentration required for 50 % maximal vessel dilation (EC_{50}) was determined for each treatment group.
Results: The OVX and OVX-E groups did not differ significantly in age (93.8 ± 1.1 days and 93.9 ± 1.1 days; p=1.0). There were significant differences in body weight (282.8 ± 11.9 mg and 227 ± 26.8 mg; p=0.007) and uterine weight (0.18 ± 0.1 mg and 0.83± 0.2 mg; p=0.008). Uterine arteries from OVX and OVX+E rats responded with dose-dependent vasodilation to increasing concentrations of ACh. Sensitivity to EDHF-mediated uterine vasodilation was significantly augmented by estrogen replacement. EC_{50} values determined for the arteries from OVX rats were significantly higher then those from OVX+E rats (2.2 ± 3.1 μM vs. 0.2 ± 0.1 μM p=0.023).
Conclusion: The resistance vessels of the rat uterus can be effectively dilated via an EDHF-mediated mechanism. Estrogen replacement results in significant augmentation of EDHF-mediated vasodilation in the uterine vessels and is therefore likely responsible for the previously documented gestational effect.
Supported by University of Vermont OBGYN Departmental Award and NIH HL067250.

486

Could Androgens Influence Human Luteal Cells Function? Rosanna Apa,*[1] Anna Tropea,[1] Fiorella Miceli,[1] Francesca Minici,[1] Mariateresa Orlando,[1] Maria F Gangale,[1] Federica Romani,[1] Federica Tiberi,[2] Stefania Catino,[3] Roberta Nestorini,[1] Antonio Lanzone.[3] [1]*Cattedra di Fisiopatologia della Riproduzione Umana, Università Cattolica del Sacro Cuore, Roma, Italy;* [2]*Istituto Scientifico Internazionale "Paolo VI", Università Cattolica del Sacro Cuore, Roma, Italy;* [3]*Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina, EN, Italy.*
Objective: In PCOS patients reproductive dysfunctions are frequently observed even if ovulation can occur. An impaired luteal function could partially explain this subfertility, since luteal steroidogenesis deficiency and premature luteal degeneration have been described in PCOS patients. Based on the frequent observation of high plasmatic levels of androgens in PCOS, in the present study we investigated whether hyperandrogenism could negatively affect luteal function. To this aim, we tested the *in vitro* effects of androgens on Progesterone (P) and on Vascular endothelial growth factor (VEGF) production by human luteal cells. Indeed, VEGF is essential for normal luteal development and function being an important regulator of angiogenesis and vascular permeability. Since Prostaglandins (PGs) play a central role in modulating luteal function, the influence of androgens on PGE_2 and $PGF_{2\alpha}$ secretion was also investigated.
Methods: Highly purified human luteal cells were cultured for 24 h with medium alone (control) or in presence of increasing concentrations (from 10^{-8} to 10^{-6}M) of testosterone or dihydrotestosterone or androstenedione. mRNA VEGF levels were evaluated by semiquantitative RT-PCR, whereas, in the culture medium, VEGF secretion was assayed by ELISA. Progesterone secretion was evaluated by ELISA, while PGs production was assayed by RIA.
Results: Our results demonstrated that testosterone, androstenedione and dihydrotestosterone were all able to significantly reduce both VEGF secretion and VEGF mRNA expression in human luteal cells. Androgens were also able to significantly decrease P and $PGF_{2\alpha}$ secretion, while preliminary results did not show any effects on PGE_2 production.
Conclusion: Increased levels of androgens were able to decrease luteal VEGF synthesis and P production. These effects might be involved in the luteal insufficiency frequently observed in women with PCOS. In this context the influence of androgens on PGs secretion requires further investigations.

487

Cyclic Regulation of Epithelial GATA-3 Expression in Human Endometrium. Danielle A Inman, Lingwen Yuan, Steven L Young.* *OB/GYN – Reproductive Endocrinology, UNC-Chapel Hill, School of Medicine, Chapel Hill, NC, USA.*
Objective: The endometrial functions of reproduction, menstruation, and host defense are regulated by locally-produced cytokines; however, the mechanisms regulating endometrial cytokine expression are poorly understood. Regulation of T-lymphocytes cytokine expression involves differentiation toward either a TH1 or TH2 phenotype, resulting in expression of different sets of cytokines. TH2 differentiation depends upon the GATA-3 transcription factor, while TH1 differentiation requires T-bet. Bias of cytokine expression away from TH1 and toward TH2 is thought to support pregnancy. Our previous work has described cyclic regulation of T-bet in endometrial epithelial cells, with maximal expression in the late secretory phase, suggesting that factors regulating T-cell expression of cytokines may also regulate cytokine expression in the endometrial epithelium.
Objective: Determined the endometrial expression of GATA-3 mRNA and protein across the menstrual cycle.
Methods: Endometrial samples were obtained, under an IRB approved protocol, from normal volunteers representing all phases of the menstrual cycle (n=18). GATA-3 mRNA was quantitated using TaqMan qRT-PCR, using the delta-delta Ct method and cyclophilin as a housekeeping gene. In addition immunohistochemistry on fixed endometrial specimens were used to localize GATA-3 protein. An isotype-matched antibody against a non-relevant antigen served as a negative control.
Results: Relative expression of GATA-3 mRNA was markedly increased (8-fold) in the late secretory phase of the menstrual cycle as compared to the early secretory phase, while expression was low during the early secretory and mid-secretory phases. GATA-3 protein localized to the cytoplasm of glandular and luminal epithelium during the late secretory phase, but was also present at low levels in the same cells during the early and mid-secretory phases.
Conclusions: GATA-3 is transcribed and translated in endometrial epithelium in a cyclic manner, with maximal expression in the late secretory phase. Coexpression of GATA-3 and T-Bet is unexpected and may be unique to endometrium.



488

Optimizing the Total Reproductive Potential in IVF and Oocyte Donation: The Elective Transfer of Two Embryos Results in High Pregnancy Rates and Minimizes Multiple Gestations. Silvina Bocca,* Laurel Stadtmauer, Sergio Oehninger.* *Obstetrics and Gynecology, The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, USA.*

Background: Multiple gestations, and their associated negative effects on maternal-fetal and perinatal health, represent a major complication of infertility treatments. The important goal to decrease multiple pregnancies in IVF might be accomplished by limiting the number of embryos transferred and/or the optimization of embryo selection methods.

Objective: The aim of this study was to assess the impact of the implementation of a 2-embryo transfer policy on conception and multiple pregnancy rates in good prognosis IVF patients and in oocyte donation (OD).

Materials: A total of 814 cycles were analyzed. Inclusion criteria: (i) IVF patients were ≤38 years and oocyte donors were <32 years; (ii) all women were stimulated with a combination of a GnRH agonist and recombinant FSH; (iii) ≥9 mature oocytes were harvested; and (iv) embryos were selected for transfer on day 3 and excess concepti of good quality were cryopreserved. Patients having a selective transfer of 2 embryos (SET2) in the fresh and subsequent frozen/thawed cycles were compared to patients having a selective transfer of 3 embryos (SET3). We hypothesized that the analysis of the total reproductive potential (TRP) of a single cycle of ovarian stimulation is optimized by including pregnancies arising from fresh and frozen-thawed embryo transfers resulting from the stimulated cycle.

Results: There were 459 fresh cycles and 355 frozen/thawed cycles, resulting in 281 pregnancies. The TRP of SET2 cycles was 63% in IVF and 53% in OD, whereas the TRP of SET3 cycles was 60% in IVF and 67% in OD (not significant). The multiple pregnancy rate was significantly lower in SET2 versus SET3 (25% vs 39%, P<0.05), with twin gestations decreasing from 31% to 23%, and triplets from 9% to 2% (P<0.02).

Conclusions: In IVF and OD, the TRP, which is considered the true estimate of the overall efficiency of a single stimulated cycle, was similar for SET2 and SET3. Importantly, SET2 significantly reduced multiple pregancies and high order multiple gestations.

489

DHT Production by Postmenopausal Ovaries: Indirect Evidence for Continued 5a-Reductase Activity? Marc J Kalan, Robin H Fogle, Liqun Wu, Richard J Paulson,* Frank Z Stanczyk.* *OB/GYN, University of Southern California, Los Angeles, CA, USA.*

Introduction/Objectives:

Serum dihydrotestosterone (DHT) production in women results from peripheral conversion of androgenic precursors by 5a-reductase. Recent studies have demonstrated that the premenopausal ovaries contain 5a-reductase mRNA and thus may be involved in DHT production. While we have recently substantiated continued androgenic activity of the postmenopausal ovary, its contribution to circulating DHT has not been documented.

Methods:

Six postmenopausal women undergoing TAH/BSO, median age 61 years (range 45-72), were recruited for participation. Menopausal status was confirmed by FSH>20 mIU/mL (median 57 mIU/mL, range 38-76 mIU/mL) and/or amenorrhea for greater than 12 mos (median 117 mos., range 9-120 mos). At the time of TAH/BSO, ovarian venous and peripheral blood was collected simultaneously. Additionally, peripheral blood was collected both preoperatively and postoperatively. Serum was analyzed for DHT using highly sensitive RIA following organic solvent extraction and Celite column partition chromatography. Analysis was performed using Wilcoxon sign-rank test.

Results:

There was a statistically significant gradient between ovarian venous and peripheral DHT levels (median (range): 60 pg/ml (34-126 pg/ml) vs 40 pg/ml (34-47pg/ml); p< 0.05). We observed that postoperative levels of DHT decreased compared to preoperative levels (45 pg/mL (28-79 pg/mL) vs 38 pg/mL (21-60 pg/mL); p>0.05). This difference however, did not reach statistical significance.

Conclusions:

1. The postmenopausal ovary continues to secrete DHT as demonstrated by a statistically significant gradient between ovarian venous and peripheral levels.
2. Postoperative concentrations of DHT did not decline significantly following removal of the postmenopausal ovary. This may be the result of a small sample size or of compensatory peripheral conversion of androgenic precursors to DHT.
3. Ovarian DHT secretion provides indirect evidence of continued 5a-reductase activity by the postmenopausal ovary.

490

Endocrinological Evaluation of Dietary Ovulatory Dysfunction in Lipid/Bone Metabolism. Kanoko Imai, Masako Tanaka, Hidenori Sasa, Noriko Makimura, Kenichi Furuya. (SPON: Toshiaki Okawa). *Obsterics and Gynecology, National Defense Medical College, Tokorozawa, Saitama, Japan.*

Objective: To study the relationship between lipid/bone metabolism and adipocytokine in the therapy for the patients with menstrual dysfunction occurred by the weight change. Methods: Twelve women with dietary amenorrhea(A group) were received the Kaufmann therapy and eight obese women(B group) were received the dietary therapy. The endocrinological function, lipid/bone metabolism and bone mineral density(BMD) were evaluated during the therapy. Leptin, adiponectin, orexin, and ghrelin were measured in the both groups with informed consent. Results: BMD, serum osteocalcin and urine N-telopeptides of type I collagen in the A group was 0.85g/cm2, 5.2±0.9ng/ml and 43.8±12.1nM/mM Cr, respectively. In the B group there are the patients with low BMD. Leptin showed positive correlation and adiponectin showed negative correlation with BMI in the both groups. Ghrelin showed negative correlation with BMI, but in the A group there are the patients with low ghrelin value. Conclusion: The evaluation in lipid/bone metabolism and adipocytokine was necessary for the endocrinological treatment of dietary ovulatory dysfunction.

491

The Kv4.3 Potassium Channel Is Expressed in Rat Hypothalamic Gonadotropin-Releasing Hormone (GnRH) Neurons and Immortalized GnRH Neurons. Armando Arroyo,*[1] Beomsu Kim,[1] Jennifer L Peresie,[1] Wh Lee,[1] Glenna CL Bett,[1,2] John Yeh.*[1] *[1]Gynecology-Obstetrics, University at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY, USA; [2]Physiology and Biophysics, University at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY, USA.*

Objective: Gonadotropin-releasing hormone (GnRH) pulsatile secretion from hypothalamic GnRH neurons is controlled by GnRH neuronal electrical activity. The role of ion channels in GnRH neuronal excitability is not clear. The major potassium (K^+) current found in GnRH neurons is the transient K^+ current also known as the A-type current (I_A). In neurons, I_A plays a crucial role in determining firing frequency and action potential repolarization. In the brain, I_A is generated by voltage-gated K^+ channels including Kv1.4, Kv3.4, and the Kv4 subunits (Kv4.1, Kv4.2, and Kv4.3). In hypothalamic GnRH neurons I_A resembles Kv4 channel currents. Thus it is possible that Kv4 may play an important role in determining firing frequency in GnRH neurons. Our hypothesis is that Kv4 channels are expressed and regulate GnRH secretion in GnRH neurons.

Methods: To determine whether Kv4.3 is expressed in rat GnRH neurons we used double-label fluorescence immunohistochemistry. RT-PCR and western blotting was used to detect Kv4.3 gene and protein expression in GT1-7 cells.

Results: We found that 83% (76/92) of rat hypothalamic GnRH neuronal cell bodies in the medial preoptic area immunostained for Kv4.3 using double-label fluorescence immunohistochemistry. GnRH axon terminals in the median eminence were negative for Kv4.3. Kv4.3 mRNA and protein was detected in GT1-7 cells using RT-PCR and western blot.

Conclusions: This study demonstrates for the first time that Kv4.3 is expressed in native GnRH neurons and GT1-7 cells. Our research supports the hypothesis that Kv4.3 potassium channels are involved in GnRH neuronal excitability and GnRH secretion in GnRH neurons.

492

Blockade of Estrogen Receptors Decreases Fetal CNS Cyclooxygenase-2 Expression. Christine E Schaub, Charles E Wood.* *Dept. of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*

Estradiol administration causes increased plasma ACTH and cortisol in the fetus. We have proposed that there is a positive feedback relationship between estrogen and fetal HPA axis activity that is dependent upon cyclooxygenase (COX) activity in the fetal brain. The present study was designed to investigate genomic and hormonal responses to blockade of estrogen receptors in the fetal CNS. Six time-dated pregnant ewes with chronically-catheterized twin fetuses were used in this study. In each pregnancy, one twin was treated icv with the estrogen receptor antagonist ICI 182,780 (25 ug/day; n=6) while the other

twin served as an age-matched control. Infusions were delivered by osmotic minipump, initiated at the time of surgery, and continued for 7-10 days. Fetuses were surgically prepared between 120-127 days gestation and sacrificed between 130-134 days gestation. Fetal blood samples were drawn for plasma hormone and blood gas analysis prior to sacrifice. Plasma hormone concentrations were measured using radioimmunoassay and immunoradiometric assay and mRNA abundances were measured using real-time RT-PCR with specific Taqman probes and primers. ICI infusion significantly decreased COX-2 mRNA abundance in fetal hypothalamus, brainstem, hippocampus, cerebral cortex, and cerebellum as well as in pituitary. Decreases in COX-2 expression were especially pronounced in hippocampus and in pituitary. There were no statistically significant changes in COX-1. ICI significantly decreased plasma concentrations of dehydroepiandrosterone sulfate (DHEAS) and progesterone relative to controls but had no statistically significant effect on fetal plasma ACTH, proopiomelanocortin (POMC), or cortisol. Analysis of mRNA revealed no statistically significant effects of ICI on arginine vasopressin, corrticotropin releasing hormone, steroid sulfatase, and serum and glucocorticoid regulated kinase-1 in hypothalamus, and POMC and prohormone convertase-1 in pituitary. We conclude that COX-2 expression in the late-gestation fetal brain is in part stimulated by circulating estrogens in fetal plasma. We also conclude that fetal DHEAS and progesterone are stimulated by an action of estrogen in the fetal brain, although these effects are not mediated by changes in fetal HPA axis activity.

493

Does Mammary Serotonin Production Impact the Central Serotinergic System. Betsy A McCormick,[1] Karen A Gregerson,[2] Nelson D Horseman.[3] (SPON: Michael A Thomas). *[1]Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA; [2]Division of Pharmaceutical Sciences, University of Cincinnati, Cincinnati, OH, USA; [3]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.*

Objective: Serotonin (5-HT) is a central neurotransmitter important in mood regulation, however, recent studies have revealed it is also produced in the mammary tissue and its expression increases during peripartum. Our objective is to determine if mammary 5-HT has effects on the central 5-HT system.

Methods: This study used a tryptophan hydroxylase 1 knock out (TPH1KO) mouse model compared to wild type (WT) mice. TPH 1 is found only in peripheral tissue, and is the rate limiting enzyme for 5-HT production, hence TPH1KO mice do not produce mammary 5-HT. Virgin and lactating mice 5-HT levels were compared. Lactating mice were normalized to 6 suckling pups/dam and were taken on the peak day of lactation. Mice underwent perfusion fixation with 4% paraformaldehyde and the brain and duodenum were removed. Immunohistochemisty was performed for 5-HT in the neural dorsal raphe at the level of the pontine nucleus and in the duodenum (major sources of 5-HT). Positive cells were quantified by counting and represented by the average of triplicate counts, and scored for staining intensity using Image J (n=4/group). Our results were evaluated by one way ANOVA with Tukey's test for differences among groups.

Results: The brains of WT virgin mice had substantially greater staining intensity and number of cells stained for 5-HT compared to all other groups. TPH1KO virgin mice had significantly less neural 5-HT compared to WT virgins ($p<0.001$, $p<0.001$ cell count and staining intensity, respectively for all data reported) and the WT lactating mice had significantly less neural 5-HT compared to the WT virgin mice ($p<0.01$, $p=0.03$). Staining for central 5-HT in the TPH1KO virgin and lactating mice were not significantly different from each other ($p=0.90$, $p=0.97$). 5-HT staining in duodenal tissues did not differ significantly between virgin and lactating mice in either genotype ($p=1.0$, $p=0.438$), but were significantly less in the TPH1KO mice.

Conclusions: Our initial data indicate that central 5-HT levels are significantly less during lactation, thus peripheral mammary 5-HT production impacts central 5-HT levels. This new evidence may lead to a better understanding of how the reproductive system can alter brain physiology, particularly how central 5-HT may be regulated peripartum.

494

Gap Junctional Communication among Cumulus Cells as a Determinant of Pregnancy Outcome in Assisted Conception. Hong-Xing Wang,[1] Dan Tong,[1] Francis R Tekpetey,[2] Gerald M Kidder.[1] (SPON: Bryan S Richardson). *[1]Physiology and Pharmacology, and Obstetrics and Gynaecology, University of Western Ontario, London, ON, Canada; [2]Obstetrics and Gynaecology, University of Western Ontario, London, ON, Canada.*

Objectives: Evidence from mutant mouse models has indicated that gap junctional intercellular communication (GJIC) among cumulus cells plays an important role in folliculogenesis since its deficiency leads to impaired fertility. Multiple connexins (Cx), the protein subunits of gap junction channels, have been found within ovarian follicles in several species. However, little is known about the identity and function of connexins in human follicles. The objectives of this study were to (1) identify connexins expressed in human cumulus cells and (2) assess the strength of that expression and of GJIC in relation to the outcome of assisted conception via intracytoplasmic sperm injection (ICSI).

Methods: Cumulus cells were removed from oocyte-cumulus cell complexes in preparation for ICSI. Expression of 20 connexin mRNAs was examined by RT-PCR and the presence of the proteins was confirmed by immunostaining. Relative amounts of the predominant connexin, Cx43, were measured by immunoblotting. Strength of GJIC was determined by fluorescent dye injection and patch-clamp electrophysiology.

Results: All but five of the 20 connexin mRNAs tested were detected. Of these, Cx26, Cx32, Cx40, and Cx43 were confirmed to be present as proteins, but Cx37 and Cx45 were not detected despite the presence of their mRNAs. Cx43 formed a large number of gap junction-like plaques between the cells, but Cx26, Cx32, and Cx40 mainly localized in the cytoplasm with only a few putative membrane plaques being found. Co-immunostaining experiments demonstrated co-localization of Cx26 and Cx32 with each other but not with Cx43. Dye injection and patch-clamp electrophysiology confirmed strong GJIC among cumulus cells of most, but not all, patients. Cx43 expression and GJIC were stronger among cumulus cells from patients who became pregnant than from those who did not.

Conclusions: These results indicate that Cx43 is a major contributor to gap junctions in human cumulus cells and that pregnancy outcome in assisted conception programs may be influenced by the level of expression of this connexin and/or the strength of GJIC among cumulus cells. *Supported by the "Healthy Gametes, Great Embryos" Strategic Initiative of the Institute of Human Development, Child and Youth Health, Canadian Institutes of Health Research.*

495

A Computational Parameter Study of Embryo Transfer. Paolo Rinaudo,[1] Sava Dashev,[2] Mitchell Rosen,[1] Milani J Albert,[2] Istvan Lauko.[2] (SPON: Marcelle I Cedars). *[1]Obstetrics, Gynecology, University of California, San Francisco, San Francisco, CA, USA; [2]Mathematical Sciences, University of Wisconsin, Milwaukee, WI, USA.*

Introduction: Embryo transfer (ET) is the final component of the in vitro fertilization process, and plays an important role to its final success rate. The procedure is however performed differently by different clinicians and overall there is a lack of formal analysis of the process. The goal of this study is to investigate what procedural parameters need to be utilized by the practitioner to optimize the process.

Material and methods: ET was studied on a 2-dimensional fluid dynamics model utilizing a multiprocessor computational platform. The governing equations were represented by the Navier-Stokes equation for an incompressible fluid with constant viscosity. We considered six parameters to determine what influences embryo placement: *injection time* (IT), *catheter distance* (CD) from the fundus, *injected volume* (IV), *rest time* (RT) before withdrawal, *withdrawal speed* (WS) and *volume replacement* (VR) i.e. the injection of a small volume of fluid during catheter withdrawal. To identify the region reached by the embryos after ET we traced an array of 7 potential embryo trajectories. Overall, 729 data points were calculated. The computations have been performed on a 9-node Linux Beowolf cluster. With dedicated usage of this platform and without the implementation of parallelization techniques, the computational experiment required over 400 hours.

Results: CD and IV strongly correlate while IT, RT and WS weakly correlate with optimal embryo placement. In particular a CD of 0.5 cm from the fundus and a small IV (20 μl) offer the best results among the different parameters studied. Catheter withdrawal is associated with a back flow of the injected fluid toward the cervix, although no embryos were expelled from the cervix. WS has

little effect on the rate of embryos movement. Because of the fluid displacement during withdrawal, we added VR in the investigation. Calculations show that replacing with fluid the volume of the withdrawn catheter results in minimal or no displacement of the embryos.
Conclusions: These calculations offer the first formal analysis of the ET process; based on this study we propose a set of optimal parameters with which to perform ET. In addition, we suggest a novel technique of volume replacement simultaneous to the catheter withdrawal. Further clinical studies will be needed to confirm the results of this study.

496

Early Serum Cytokine Evaluation May Prove Useful in Localizing Abnormally Implanted Human Gestations. Sara Morelli, Debbra Keegan, Lewis C Krey, Joseph Katz, Mengling Liu, Nicole Noyes. (SPON: Mortimer Levitz). *Obstetrics and Gynecology, NYU Fertility Center, New York, NY, USA.*
Objective: To determine whether early measure of serum cytokines (IL-2R, IL-6, and IL-8) along with hCG and progesterone (P4) can differentiate an ectopic (EG) from an intrauterine gestation (IUG).
Methods: A retrospective analysis of IVF cycles from 2000-2005 performed at a university-based fertility center. The study groups: EG (sac identified outside of the uterus on ultrasound (n=15), spontaneous abortion (SAB) (n=30) or singleton term delivery (TD) (n=30). Cytokine assays were run on frozen-thawed serum samples collected 2 (D28) and 3 (D35) weeks post-oocyte retrieval using an automated, immunoassay analyzer. Previously measured hCG and P4 levels from the same serum samples were evaluated. Receiver operating characteristic curves were constructed.
Results: Average hCG levels on D28 and D35 were higher in cycles ending in TD than in all other pregnancy groups ($p \leq .003$); an hCG level >94 mIU/mL on D28 and >1372 mIU/mL on D35 predicted a live birth outcome with a sensitivity and specificity of 80%. In the setting of a rise or plateau in hCG value, median hCG readings on D28 and D35 were significantly lower in the EG group than in than in those with SAB or TD ($p<.001$); an hCG level <37 mIU/mL on D28 and <637 mIU/mL on D35 distinguished an EG from a SAB and TD with a sensitivity and specificity ≥85%. On D28, median IL-8 levels were lower in the EG group when compared to all IUGs combined ($p<.04$). Contrary to prior reports, no significant differences in IL-2R and IL-6 levels were noted between groups. All patients received 50 mg IM P4 supplementation. Even so, median D35 P4 levels were lower in EG than in SAB and TD cycles (D35: $p<.001$); a D35 P4 level <35 ng/mL distinguished an EG from an SAB and TD with a sensitivity of 80% and specificity of 87%.

Median serum marker levels

	IL-8 (pg/mL)		hCG (mIU/mL)		P4 (ng/mL)	
	D28	D35	D28	D35	D28	D35
EG	6.1[a]	5.3	20	329	35.8	29.6[d]
SAB	8.3[b]	5.6	75.5	1296.5	53.6	66.3[e]
TD	7[c]	6.6	116	2094	52.6	71.7[f]

a-b: $p<0.01$; b-c: $p<0.05$; d-e, d-f : $p<0.001$; *Difference in hCG between all groups: $p \leq 0.003$.

Conclusions: The absolute D35 serum hCG level was the single most predictive measure in early pregnancy. In the setting of a rise or plateau in hCG levels, low D28 IL-8 and D35 P4 levels (despite P4 supplementation) suggested an extrauterine implantation. This assay combination may facilitate earlier diagnosis of an EG when pregnancy location is unclear.

497

Anti-Mullerian Hormone Used To Assess Ovarian Function in SLE Patients Presenting for Hematopoietic Stem Cell Transplant. Hyacinth Browne, Alicia Armstrong, Rebecca Babb, James Segars,* Steven Pavletic. *Reproductive Biology and Medicine Branch, NICHD, and NCI, National Institutes of Healtlh, Bethesda, MD, USA.*
Objective: To evaluate ovarian function among SLE patients undergoing hematopoietic stem cell transplant (HSCT) using anti-mullerian hormone and current markers of ovarian reserve. **Materials and Methods**: A retrospective analysis from a phase II study of 6 patients with Stage IV lupus nephritis, evaluated at the NIH as part of an autologous HSCT protocol, were reviewed between January 2004 and October 2006. Information was abstracted for menstrual and reproductive history, and the use of hormonal medications prior to HSCT. Measures of ovarian reserve included age, menstrual function, FSH, estradiol, and AMH levels measured at 4 and 6-9 months post-treatment.
Results: Patients received a mean of 14 cycles of IV cyclophosphamide prior to HSCT. Three patients received hormonal therapy-prior to HSCT. Two patients were menopausal and one pre-menarchal at the start of the study, and did not receive hormonal treatment. One patient expired, one was lost to follow-up, and one patient was only 4 months post-transplant. All patients ≥ 31 years of age had FSH levels in the menopausal range prior to hematopoietic stem cell transplant. Two patients with premenopausal FSH and estradiol levels had regular menses prior to their diagnoses, and subsequently became amenorrheic after receiving IV cyclophosphamide. Patient with regular menses had higher AMH levels. Patients who had amenorrhea and somatic symptoms consistent with ovarian failure had low AMH levels. One patient who developed amenorrhea had normal FSH levels, in the presence of low AMH levels.

Age	Age at Dx	# cycles	Baseline FSH (U/L)/ E2 (pg/ml)	4 months FSH (U/L)/ E2 (pg/ml)	6-9 months FSH (U/L)/ E2 (pg/ml)	4 months AMH (ng/ml)	6-9 months AMH (ng/ml)
19[A]	19	5	3/<20	8/33.7	6/35.9	0.4	0.15
20[B]	14	19	4/28.3	8/33.8	7/37.1	<0.1	
38[CD]	32	16	71/46.4	49/30.3	53/34.4		
16	14	10	3/23.7	7/57.6	4/94.3	0.6	0.5
32[C]	26	22	110/31	96/<20	129/<20	<0.1	<0.1
30[B]	20	8	14/<20	49/31.5		<0.1	

[A] Depot Provera, [B]GnRHa, [C]Menopausal prior to HSCT, [D]Expired during study
Conclusion: The risk of sustained amenorrhea related to both age at IVCY initiation and the number of IVCY cycles. AMH levels correlated more with the rate of sustained amenorrhea than FSH and estradiol levels, and may facilitate earlier identification of decreasing ovarian reserve.

498

Clomiphene Citrate Pregnancy Outcomes for over 3,500 Cycles. Serena Dovey,[1] Rita M Sneeringer,[1,2] Alan Penzias.*[1,2] *[1]Ob/Gyn, Reproductive Endocrinology & Infertility, Beth Israel Deaconess, Boston, MA; [2]Boston IVF, Waltham, MA.*
INTRODUCTION: Although clomiphene citrate (CC) is a standard first-line treatment for infertile couples, reported efficacy varies widely within the published literature. Additionally, efficacy of CC is thought to differ based on infertility diagnosis, with CC being most effective for the anovulatory population. The aim of this study was to evaluate the overall pregnancy rate with CC in a generalized infertility population using the largest outcomes data for CC to date.
MATERIALS / METHODS: A retrospective data analysis of all infertile patients treated with CC at the Boston IVF centers from September 2002 through July 2006 was performed. Pregnancy rate (including live births and losses) for 3,899 CC cycles was evaluated. Pregnancy outcomes were compared among subgroups divided based on age.
RESULTS: The overall pregnancy rate was 8.7% per cycle (339 pregnancies for 3,899 total cycles). An inverse relationship between age and success rate was noted (Table 1). For women under 35 years, a pregnancy rate per cycle of 11.3% was seen. In comparison, the pregnancy rate per cycle for women between age 41 and 42 years and over age 43 years was 4.6% and 1.1%, respectively. The mean patient age in this study was 34.8 years, and ranged from 20 to 48 years. SPSS statistical software was utilized for pregnancy rate analysis.
CONCLUSIONS: CC efficacy in achieving a pregnancy approximated 9% per cycle. However, as with many advanced reproductive technologies (ART), advancing age dramatically reduced the mean pregnancy rate. Given that this is the largest data series to date for CC success in a generalized infertility population, it should prove helpful in counseling women regarding outcomes who initiate treatment with CC.

CC Pregnancy Rate Per Cycle

Age (years)	<35	35-37	38-40	41-42	>42
Number of Patients	2192	885	571	150	101
Number of Pregnancies	224	71	37	6	1
Pregnancy Rate per Cycle (%)	11.3	8.7	7.1	4.6	1.1

A reduction in efficacy of CC is noted with advancing age.

499

Non-Invasive Metabolomic Profiling of Human Embryo Culture Media Using Proton NMR Correlates with Pregnancy Outcome. Emre Utku Seli,*[1] Denny Sakkas,[1] Richard Scott,*[2] Lucy Botros,[3] David H Burns.[3] *[1]Obstetrics and Gynecology, Yale University, New Haven, CT, USA; [2]RMA of NJ, Morristown, NJ, USA; [3]Department of Chemistry, McGill University, Montreal, QC, Canada.*
Objective: More than 100,000 assisted reproductive technology (ART) cycles are started yearly in the U.S. A mean number of 3.1 embryos are transferred in ART cycles using fresh nondonor oocytes. This leads to a 34.3% overall pregnancy rate, 29.0% of which result in multiple-infant live births. These statistics reflect a need for improvement over our current embryo selection methodology that is based on cleavage rates and morphology. In this study, we

FRIDAY

hypothesized that, embryos that result in pregnancy may be different in their metabolomic profile compared to embryos that do not, and that the difference may be detected by the non-invasive evaluation of the embryo culture media using proton nuclear magnetic resonance (NMR).
Materials and Methods: Embryo culture medium of 61 embryos from ART cycles using fresh donor or nondonor oocytes were evaluated. Media (G1.3; VitroLife, Sweden) were individually collected after embryo transfer on day 3, and analyzed using proton NMR. The spectra obtained was analyzed using a ppm selective genetic algorithm (GA) to determine regions predictive of pregnancy outcome as determined by logistic regression. To avoid random correlations, a leave-one out cross-validation was used. Sensitivity and specificity of predicting pregnancy (defined as presence of fetal cardiac activity) were calculated.
Results: Spectral profile describing differences in lactate, alanine, and glycine concentrations showed distinct differences between culture media of embryos that resulted in pregnancy and those that did not. Using GA to select spectral regions associated with these molecular species, a model predictive of pregnancy outcome was developed. Compiled outcomes from the leave-one-out cross-validation of the logistic regression using the NMR measurements resulted in a specificity of 84% and a sensitivity of 86%.
Conclusion: In this study we observed a difference in the metabolomic profiles of embryo culture media obtained from embryos that cause pregnancy compared to those that do not. Our findings suggest that metabolomic profiling may serve as a useful methodology for non-invasive embryo assessment and selection. Confirmation of these initial observations is planned through a larger prospective study involving additional ART centers.

500

Embryo Transfer Experience of Canadian REI Fellows. Jason A Hitkari,[1] Michael D Wittenberger,[2] Toni Di Berardino,[1] Ellen Greenblatt.[1] (SPON: Peter von Dadelszen). *[1]Reproductive Biology Unit, Mt. Sinai Hospital, Toronto, ON, Canada; [2]Reproductive Biology Medicine Branch, NICHD/NIH, Bethesda, MD, USA.*
Recently, information on the embryo transfer (ET) experience of U.S. REI Fellows and recent graduates demonstrated that even though the majority of respondents felt ET training was important, only 55% performed an ET during Fellowship. Information on the ET experience and attitudes of Canadian REI Fellows is currently not available. It is also not currently known whether allowing Fellows' to perform ET's results in overall lower pregnancy rates for a particular clinic.
OBJECTIVES
1. To characterize the ET experience of Canadian REI Fellows
2. To compare pregnancy rates (PRs) of Fellows to Attending physicians
3. To determine if PR's improved with increasing Fellow experience
METHODS
1. Cross-sectional survey of current REI Fellows
2. Retrospective analysis of PR's of Fellows versus Attendings (Mt.Sinai Hospital,Toronto; 2002-2005)
3. Retrospective analysis of PRs from Fellows' 1st 25 ETs versus their 2nd 25 ETs
RESULTS
All eight of the surveys sent were returned. All respondents had performed ETs during their first year of Fellowship training. All "second year" respondents had completed more than 50 ET's. Respondents felt that ET training during Fellowship was "extremely important" (87.5%) or "important" (12.5%).
No statistically significant difference was identified between the PRs from Fellows versus Attendings (Table 1). No statistically significant difference was found between the PR from Fellows' first 25 ETs and ETs 26-50 (Table 2).
CONCLUSIONS
1. All current Canadian REI Fellows perform ETs starting in the first year of Fellowship
2. ET experience is important to REI Fellows
3. Allowing Fellows to perform ETs does not appear to have a detrimental effect on PRs
4. The skills needed to perform ETs with current technology are likely acquired rapidly.

Table 1. Combined PR for all Fellows compared with the combined PR for the three Attending physicians (2002-2005; Mt.Sinai Hospital, Toronto).

	No. Of Physicians	PR/ET	Student's t-test
Fellows	7	223/749 = 29.8%	t = 0.182
Attendings	3	177/580 = 30.5%	

Table 2. The combined PR for the first 25 ET's compared with the combined PR for the second 25 ET's for five fellows from 2002-2005 who completed 50 or more ET's.

	PR/ET	Student's t-test
First 25 ETs	33/125 = 26.4%	t = 0.687
Second 25 ETs	38/125 = 30.4%	

501

Single Day and Multiple Day Pharmacokinetics of a Novel Vaginal Micronized Progesterone Tablet (Endometrin®) Compared to Crinone® Vaginal Gel in Healthy Female Subjects. Richard A Preston,[1] Paul M Norris,[2] Emily J Blake,[3] Vladimir I Yankov.[3] (SPON: John L McGuire). *[1]Department of Medicine, Miller School of Medicine, Miami, FL; [2]Department of Obstetrics and Gynecology, Miller School of Medicine, Miami, FL; [3]Clinical Research and Development, Ferring Pharmaceuticals, Inc, Suffern, NY.*
Objective: Progesterone for vaginal administration is currently available as a vaginal gel (Crinone™ 8%) for the treatment of infertile women with progesterone deficiency. A novel effervescent vaginal micronized progesterone tablet (Endometrin®) is currently under development. The purpose of this study was to determine the pharmacokinetic (PK) profiles of 2 dosage regimens of vaginal micronized progesterone tablet (100 mg BID and 100 mg TID) compared to 90 mg QD of the vaginal gel.
Methods: Single-center, randomized, open-label, Single-Day, and Multiple-Day (5 days) PK study in healthy reproductive age subjects 18-40 years old with intact uterus. 6 subjects per group received either vaginal tablet 100 mg BID or 100 mg TID or 8% gel (90 mg QD).
Table 1. Results (Data are mean values of calculated PK parameters)
All enrolled subjects completed the study. Both vaginal tablets and vaginal gel were generally safe and well tolerated. All AE were mild in intensity.
Conclusions: Tablet formulations reached higher Cmax, produced greater systemic exposure (AUC0-24), and achieved steady state more rapidly than did the vaginal gel QD. Both tablet regimens, but not the gel formulation, produced trough serum progesterone concentrations above 10 ng/mL, a threshold associated with normal mid-luteal function.

Mean Values of calculated PK parameters

	Day 1 Cmax (ng/mL)	Day 1 Trough (ng/ml)	Day 1 AUC 0-24 (ng*hr/mL)	Day 5 Cmax (ng/L)	Day 5 Trough (ng/ml)	Time to steady state (hr)
BID (n=6)	17.0	13.02	217	18.6	11.5	12
TID (n=6)	19.8	9.04	284	24.1	18.06	32
Gel QD (n=6)	6.8	1.05	80.9	14.3	9.55	>120
P-value BID vs. Gel	0.01	<0.0001	0.04	0.21	0.52	
P-value TID vs. Gel	0.002	0.0018	0.005	0.009	0.11	

502

Selective Salpingography and Tubal Catheterization in an Infertile Population Reduces the Diagnosis of Tubal Occlusion and Minimizes Tubal Surgery but Does Not Improve Spontaneous Pregnancy Rates. Pratibhasri A Vardhana,[1] Michael Guarnaccia,[*1] James Silberzweig,[2] Mark V Sauer.[*1] *[1]Obstetrics and Gynecology, Columbia University Medical Center, New York, NY, USA; [2]Radiology, Columbus Circle Radiology, New York, NY, USA.*
Background:Tubal disease is a common diagnosis in women undergoing infertility treatment. Hysterosalpingography (HSG) is utilized to evaluate tubal patency. Proximal (unilateral or bilateral) tubal occlusion can be due to tubal spasm or adhesions. Selective salpingography and tubal catheterization during HSG can better assess tubal patency, and minimize unnecessary tubal surgery. Although some patients achieve pregnancy spontaneously after catheterization, many will fail conservative treatment and require *in vitro* fertilization (IVF). We present data from our center on a cohort of infertile patients undergoing HSG with tubal catheterization. **Objective**:To present diagnostic and therapeutic findings and fertility outcomes after tubal catheterization in an infertile population. **Design**:Retrospective cohort study **Patients**:22 infertile women (age 39.18 ± 4.16) with tubal occlusion **Hypothesis**:Selective salpingography and tubal catheterization reduces the diagnosis of tubal occlusion and minimizes surgical intervention, but does not increase spontaneous pregnancy rates. **Methods**:22 infertile patients underwent HSG with tubal catheterization for the evaluation of tubal patency. Tubal obstruction was defined as persistent occlusion despite tubal catheterization. Pregnancy was defined as having a positive beta-hCG hormone level. **Results**:Unilateral or bilateral proximal tubal disease was identified in all patients at HSG. 16/22, or 72% of patients were found to have patent tubes after tubal catheterization. 6/22, or 27% of patients with proximal occlusion, had persistently abnormal tubes despite catheterization. 10/16, or 63% of patients with patent tubes after catheterization required IVF and 5 of these patients achieved pregnancy after IVF. 2/16, or 12.5% of patients with patent tubes after catheterization achieved pregnancy

spontaneously. **Conclusions:**Although selective salpingography and tubal catheterization are useful in demonstrating tubal patency, reducing the diagnosis of tubal disease, and minimizing surgery in infertile patients, establishing patency after therapeutic catheterization does not improve spontaneous pregnancy rates. Moreover, many of these patients will require IVF to achieve pregnancy.

503

Is There a Benefit of Freezing at the Blastocyst Stage Compared to the Pronuclear Stage for Frozen Embryo Transfer Cycles? Betsy A McCormick, Melissa A Heidi, Michael A Thomas,* Jared C Robins, Mira Aubuchon, Daniel B Williams. *Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

Objective: The current literature investigating optimal stage for embryo freezing and transfer are conflicting. Early freezing at the 2PN stage allows for freezing of an unselected group, increasing the chances for cryopreservation. Blastocyst freezing provides a self-selection process for high quality embryos, however the total number cryopreserved may be decreased. Our objective was to compare embryo survival and pregnancy/live birth rates from frozen 2PN vs. blastocyst stage embryos in patients undergoing frozen embryo transfers (FET).

Methods: A retrospective chart review from January 2004 to September 2006 was conducted evaluating all FET cycles. Our current freezing protocol involves freezing at the 2PN stage in patients with ≥ 9 2PN's and/or at the blastocyst stage. Information was collected regarding patient demographics and FET outcomes. All values are reported as mean ± SD. Statistically significant comparisons were assessed at $p < 0.05$ utilizing Student's t-test, Fisher's exact test, and chi square.

Results: A total of 102 FET cycles were evaluated: 69 were frozen at the 2PN stage and 33 at the blastocyst stage. The 2 groups did not differ in terms of age, smoking status, gravidity, parity, BMI, use of IVF/ICSI, or infertility diagnoses. However, in the 2PN group, significantly more oocytes were retrieved (20.5 ± 6.6 vs. 16.2 ± 5.8, $p<0.01$) and frozen (8.0 ± 4.4 vs. 3.4 ± 2.2, $p<0.01$). Additionally, a greater number of embryos were thawed (4.1 ± 1.7 vs. 2.5 ± 1.5, $p<0.01$) in the 2PN group, however no difference was seen in the percentage survival between the groups. More embryos were transferred (2.6 ± 0.6 vs. 1.8 ± 0.8, $p<0.01$) in the 2PN group, but the implantation, pregnancy, and multiple rates were not different. There was no significant difference in live birth rate between the 2PN and the blastocyst group (31.1% vs. 35.5%, respectively).

Conclusions: Freezing at either the 2PN or blastocyst stage appears to confer similar outcome results. Therefore, decisions concerning the freezing of embryos at either stage will depend on patient desire, freezing related costs, or storage capabilities of the IVF center.

504

Preovulatory (PO) Predictors of Midluteal Progesterone (ML P4) Levels in Ovulation Induction (OI) Cycles. Fang-Yu Chao, Sunjung Park Kang, Shao-Ping Weng, TC Jackson Wu.* *Dept. of Obstetrics & Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Objective:

To study if any PO factor affects P4 levels in luteal phase in OI cycles.

Methods:

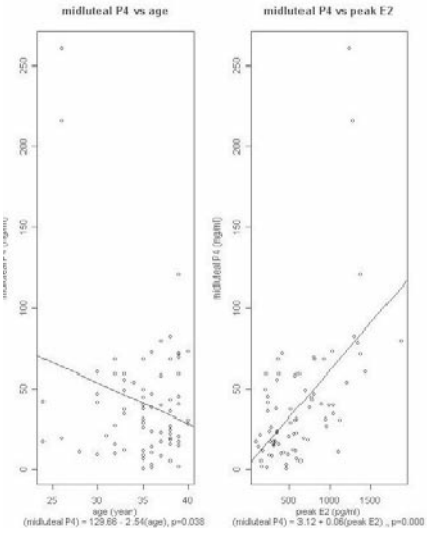
In 84 OI cycles with intrauterine insemination or timed intercourse(IUI/TIC), age, day 3 serum E2 and FSH levels, No. of mature follicles(MF, >14mm) and endometrium(EM) thickness by ultrasonography, peak serum P4 and E2 levels on the day of human chorionic gonadotropin injection, and serum ML P4 level 7 days after IUI/TIC were measured and analyzed by Fishers' exact test and regression method.

Results:

1. Controlling other factors, multiple regression showed age correlates negatively(p=.01) and peak E2 level correlates positively(p=.00) with ML P4 level.

Predictors of ML P4

Factor	P-value
Age	.01
D3 E2	.05
D3 FSH	.26
No. of MF	.67
Peak E2	.00
Peak P4	.09
EM thickness	.84



2. Increased patients' age is linked to low ML P4 level(p=.04). ML P4 level is higher in women with age <35 than age 35-40 and age >40 (64±19, 35±4 and 34±7ng/ml). Of the women with age >40, ML P4 levels are ≤30ng/ml in 60% and ≤25ng/ml in 50%.

3. Having peak E2 level>300pg/ml hold higher odds of ML P4 level>30ng/ml (odds ratio=3.3, p=.03). All women with peak E2 level<200pg/ml have ML P4 level≤25ng/ml.

4. Number of MF alone correlates with ML P4 level. ML P4 level is higher in cycles with >3 MF(76±17ng/ml) than No. of MF=1, 2 or 3(18±5, 26±5 and 35±9ng/ml, P<.01).

Peak E2, No. of MF and ML P4

	n(%)		
	ML P4 (ng/ml)		
Peak E2 (pg/ml)	**>30**	**≤30**	**N**
>300	34(59)	24(41)	58
≤300	5(29)	12(71)	17
No. of MF	**>30**	**>25**	
1	3(23)	3(23)	13
2	11(42)	11(42)	26
3	7(50)	11(79)	14
>3	18(86)	19(90)	21

Conclusions:

1. Patient's age, peak E2 levels, and No. of recruited follicles are predictive of ML P4 levels.

2. Women with age >40 is prone to low ML P4 level. ML P4 measurement and progesterone supplementation is recommended.

3. Peak E2 level >300pg/ml predicts adequate luteal support in OI cycles. Progesterone supplementation should be considered in cycles with peak E2 levels ≤200pg/ml.

4. Over 50% of the OI cycles with 1 or 2 follicles >14mm have low ML P4 levels. These patients may require progesterone supplementation.

505

Understanding the Follicular Fluid Endocrine Microenvironment. UI Ezeh, MP Rosen, AT Dobson, P Rinaudo, S Shen, Marcelle Cedars.* *Department of Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA.*

Objective: During IVF, several ovarian stimulation protocols may be used, including long lupron, microdose flare and antagonist. A change in stimulation protocol, in the same individual, affects IVFoutcome (ASRM 2006). This variation in response may be due to systemic and/or local changes in the endocrine environment during ovarian stimulation and oocyte development. Understanding the endocrine basis of variations in stimulation protocols may lead to the design of more effective protocols. Our objective is to characterize follicular fluid hormones in different stimulation protocols.

Design: Prospective cohort study.

Materials and Methods: 80 patients undergoing IVF by one of 3 stimulation protocols were studied. Follicular aspirates were obtained from 1-2 follicles measuring >15mm at the time of oocyte retrieval. Follicular fluid estradiol (E2), progesterone (P), total testosterone (T), prolactin (PRL), hCG and FSH were measured by chemiluminescent immunoassay. One-way analysis of variance was used to compare the mean values of various hormones in each protocol.

Results: Long-lupron protocol produced the lowest follicular levels of progesterone. Microdose flare produced higher amounts of progesterone than

FRIDAY

long lupron or antagonist protocols. Although antagonist produced higher progesterone levels than long lupron protocol, the difference was not significant. We also noted higher FSH levels using microdose flare than long lupron or antagonist protocols.

Conclusion: Higher follicular fluid progesterone in the microdose flare protocol may adversely affect oocyte development. Further studies are underway to evaluate the impact of follicular progesterone on individual oocyte health as reflected by fertilization rate and embryo quality and to correlate systemic and follicular endocrine profiles with the different stimulations to better understand the mechanism of this finding.

Follicular Fluid Hormones

	Long Lupron		Microdose Flare		Antagonist		
Hormones	Number of observations	Mean	Number of observations	Mean	Number of observations	Mean	**P-Value**
E2 (ng\ml)	47	498.55	9	660.00	6	627.45	0.133
FSH (IU\L)	58	5.34 a	15	11.76 b	9	7.01 c	0.000
P (ug\ml)	53	9.87 e	14	15.91 f	8	15.23 g	0.001
PRL (ng\ml)	58	41.36	15	45.00	8	25.81	0.188
hCG (IU\L)	58	84.40	15	93.93	9	77.98	0.721
T (ng\dl)	58	635.81	15	685.00	8	569.13	0.766

p< 0.05 (a vs b and b vs c; e vs f).

506

Midluteal Serum Estradiol and Progesterone Levels Determine Pregnancy Outcomes in Ovulation Induction Cycles. Sunjung Park Kang, Fang-Yu Chao, TC Jackson Wu.* *Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Objective: To evaluate the role of midluteal (ML) estradiol (E2) and progesterone (P4) levels in determining pregnancy outcomes in ovulation induction (OI) cycles

Methods: In patients undergoing intrauterine insemination / timed intercourse with OI, ML E2 and P4 levels were measured 7 days after ovulation. Clinical pregnancy was confirmed sonographically by the presence of the fetal heart beat. Biochemical pregnancy was confirmed by a positive human chorionic gonadotropin drawn 14 days after ovulation. Student's t-test, Fisher's exact test and correlation analysis were used. A value of $p < 0.05$ was considered statistically significant.

Results:

1. There was no difference in mean ML E2 levels between pregnant and non-pregnant cycles. The clinical pregnancy rate in patients with E2 ≥ 250pg/ml was 2.8 times of that of the other group with E2 < 250pg/ml, although it was not statistically significant.

2. There was a trend that mean ML P4 level in pregnant cycles was higher than that of non-pregnant cycles, although it was not statistically significant. Using P4 cut-off at 30ng/ml, there was a significant difference in the clinical but not biochemical pregnancy rate. When P4 level was less than 16ng/ml, no pregnancy was observed.

	Clinical preg (%)	Non-preg (%)
P4 < 30ng/ml	2 (4.8)	40 (95.2)
P4 ≥ 30ng/ml	8 (19.5)	33 (80.5)
	p=0.040	

3. In cycles with ML E2 < 250pg/ml and P4 < 30ng/ml, there was no clinical or biochemical pregnancy. Both the clinical and biochemical pregnancy rates were significantly higher in cycles with either E2 ≥ 250pg/ml or P4 ≥ 30ng/ml.

	Clinical preg (%)	Biochemical preg (%)	Total
E2<250pg/ml & P4<30ng/ml	0 (0)	0 (0)	27
E2≥250pg/ml &/or P4≥30ng/ml	10 (18)	13 (23.2)	56
	p=0.015	p=0.004	

4. There was a positive correlation between the ML E2 and P4 levels.

Conclusions:

1. In OI cycles, high ML E2 and P4 levels have significant impacts on pregnancy outcome.

2. Although our study indicates that ML P4 ≥ 30ng/ml is a better indicator than ML E2 ≥ 250pg/ml in predicting pregnancy outcome, E2 < 250pg/ml and P4 < 30ng/ml is an extremely sensitive indicator for non-pregnancy.

3. The correlation between ML E2 and P4 levels explains the importance of using both levels as predictors for pregnancy outcomes.

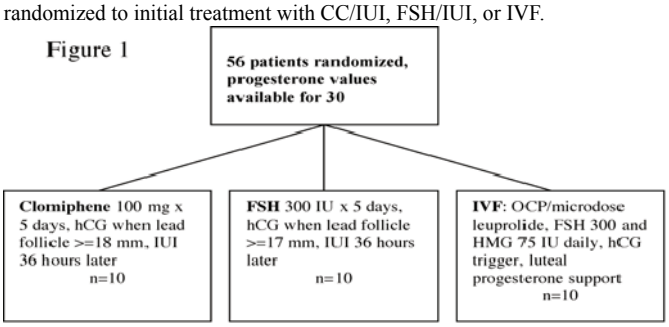
4. Whether E2 and P4 supplementation improves the clinical outcome remains to be studied.

507

Serum Progesterone Levels Do Not Differ in Older Women Undergoing Ovulation Induction with Clomiphene Citrate, Superovulation, or In Vitro Fertilization: Baseline Data from the FORT-T Trial. Tiffany A Von Wald,[1,3] Marlene B Goldman,[2] Kim L Thornton,[1,3] Richard H Reindollar.*[2] *[1]Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston, MA, USA; [2]Obstetrics and Gynecology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA; [3]Boston IVF, Waltham, MA, USA.*

Objective: The purpose of this study was to determine if serum progesterone levels differed in women undergoing ovulation induction with clomiphene citrate/intrauterine insemination (CC/IUI), superovulation/insemination (FSH/IUI), or in vitro fertilization (IVF).

Methods: Data are from the on-going FORT-T trial, a trial in which women are randomized to initial treatment with CC/IUI, FSH/IUI, or IVF.



Under IRB approval, women aged 38-43 years (n=56) were enrolled who met the following inclusion criteria: 6 months of infertility, normal semen analysis, normal uterine cavity, at least one patent fallopian tube, menstrual cycle length 21-45 days, normal ovarian reserve, and normal TSH and prolactin. Serum progesterone was measured 7-10 days after IUI or oocyte retrieval. First-cycle progesterone values for 30 of the 56 patients were compared. Statistical analysis was performed using Student's t-test. Statistical significance was set at $p < 0.05$.

Results: The mean progesterone levels (and ranges) in the CC/IUI, FSH/IUI, and IVF groups were 20.32 ng/ml (6.84-40), 13.26 ng/ml (0.77-40), and 12.71 ng/ml (7.77-23.6) respectively. There was no significant difference in progesterone levels when comparing the three treatment groups (CC vs. FSH/IUI, p = 0.21, CC vs. IVF p=0.06, FSH/IUI vs. IVF, p =0.90). There was no statistically significant difference in mean progesterone levels in women aged 41-43 years (n=28) compared to women aged 38-40 years (n=28) (p=0.95).

Conclusion: While the number of patients currently enrolled is small, this study gives us prospective, preliminary evidence that luteal progesterone values are similar in patients treated with clomiphene, superovulation, or in vitro fertilization at an older reproductive age. This finding is present despite luteal progesterone support being given to the patients randomized to IVF.

508

Second Anonymous Oocyte Donors Should Be Considered in Couples Who Have Failed with a Prior Oocyte Donor. Susan BA Hudson, David L Walker, Laura L Tatpati, Dean E Morbeck. (SPON: Charles C Coddington). *Division of Reproductive Endocrinology & Infertility, Mayo Clinic, Rochester, MN, USA.*

Objective: Anonymous oocyte donation (AOD) yields high pregnancy rates for patients who otherwise would be unlikely to conceive. Still, many recipients fail to conceive with their initial AOD cycle. We chose to analyze consecutive IVF AOD cycles of recipients utilizing a new oocyte donor following failed IVF to characterize indicators of success after failed oocyte donation cycles.

Method: IRB approved retrospective analysis of AOD IVF cycles with a 1st donor that failed to yield a pregnancy compared to AOD cycles in the same recipients utilizing a 2nd donor.

Results: Baseline characteristics and outcome measures were analyzed for the two groups. Comparison of the 1st AOD cycle to the 2nd cycle with a new donor revealed no difference in mean donor age (28.9 ± 3.7 vs 27.7 ± 2.8, p=0.45). Analysis of retrieval and transfer outcomes are found in Table 1 and 2, respectively. Six of the 9 patients delivered on their 1st transfer with the 2nd donor. One patient delivered on the 2nd transfer with the 2nd donor.

Conclusions: AOD recipients who fail to deliver with an initial donor have a high chance of conceiving utilizing a 2nd donor. This occurs despite homogeneity of characteristics with the exception of improved fertilization rates and, thereby,

increased number of fertilized oocytes. This data supports encouragement of couples who have failed with a donor to consider a 2nd attempt with a new donor. A relatively low yield of embryos with one donor can potentially be overcome by switching to a new donor.

Table 1

Retrieval Outcomes	1st Donor (N=9)	2nd Donor (N=9)	p
E2-date of hCG*	1682 (433)	1901 (630)	0.40
Oocytes Retrieved*	11.2 (5.0)	14.1 (6.0)	0.28
Mature Oocytes*	8.8 (4.0)	11.6 (4.9)	0.21
Oocytes Inseminated*	9.8 (3.8)	12.4 (4.5)	0.19
Fertilized Oocytes*	5.6 (3.0)	9.3 (4.2)	0.045
Fertilization rate (SD)	53.3 (0.2)	75.1 (0.2)	0.013

*Mean (SD)

Table 2

Transfer Outcomes	1st Donor (N=9)	2nd Donor (N=9)	p
Embryos thawed*	2.8 (1.1)	2.9 (0.9)	0.87
Embryos survived*	2.7 (0.9)	2.8 (0.9)	0.66
Embryos transferred*	2.6 (0.8)	2.5 (0.7)	0.71
% Good quality transferred (SD)	36.7 (0.4)	35.4 (0.4)	0.91
Embryo grade*	1.1 (0.5)	1.0 (0.5)	0.36
Cell #*	5.5 (1.3)	5.6 (1.6)	0.93
1st Live Birth N (%)	0	7/9 (77)	0.002
# of transfers*	1.9 (0.8)	2.7 (1.0)	0.085

*Mean (SD)

509

Intra-Cytoplasmic Sperm Injection (ICSI) Improves Fertilization Rates at In Vitro Fertilization (IVF) in Patients with Polycystic Ovarian Syndrome (PCOS). Kristen Page Wright,[1] Peter Casson,[*1] Sue L O'Brien,[1] Julia V Johnson,[*1] Takamaru Ashikaga.[2] *[1]Vermont Center for Reproductive Medicine, Fletcher Allen Health Care, University of Vermont, Burlington, VT, USA; [2]Biostatistics & Bioinformatics Facility, University of Vermont, Burlington, VT, USA.*

Objective: To determine if IVF/ICSI improves fertilization rates in patients with PCOS compared to conventional IVF. Patients with tubal factor infertility undergoing conventional IVF or IVF/ICSI were used as a control group.

Methods: A retrospective cohort of patients with PCOS undergoing IVF for infertility were compared with a cohort of patient with PCOS undergoing IVF/ICSI for male factor infertility. Patients with tubal factor infertility were used as a control group because the ICSI procedure could improve fertilization rates irrespective of the infertility diagnosis. Fertilization rate was calculated as the proportion of normally fertilized embryos, ascertained by the presence of two pronuclei on the day following insemination, divided by the number of mature oocytes retrieved. Implantation rate was defined as the number of gestational sacs present on 6 week ultrasound divided by the number of embryos transferred. Positive pregnancy outcome was defined as the presence of fetal heart tones on ultrasound. Statistical analysis was performed using a two-way analysis of variance and multiple comparisons of group means was conducted using a Fisher's least significant difference approach.

Results: There were 23 cycles of PCOS patients undergoing IVF and 18 undergoing IVF/ICSI. There were 94 cycles of tubal factor patients undergoing IVF and 36 undergoing IVF/ICSI. There was a statistically significant difference in fertilization rate between PCOS patients undergoing IVF (51.3%) compared to IVF/ICSI (68.1%, p=0.03). In contrast, there was no difference in fertilization rate in tubal factor patients undergoing IVF (72.6%) compared to IVF/ICSI (66.2%, p=0.1). Analysis of variance demonstrated that the impact of the procedure (IVF or ICSI) differed depending upon the diagnostic group (PCOS or tubal factor, p=0.012). The two diagnostic groups did not differ for the ICSI procedure (p=0.79) but they were different for the IVF procedure (p<0.001). PCOS patients undergoing IVF or ICSI had no difference in implantation rates (12.0% vs. 15.2%, p=0.93) or pregnancy rates (40.0% vs. 44.4%, p=1.00).

Conclusions: The use of ICSI improves fertilization rates in patients with PCOS undergoing IVF.

510

Naltrexone Treatment in Clomiphene Resistant Women with Polycystic Ovary Syndrome. Mohamed I Mohamed,[1,2] Antoni J Duleba,[*1] Osama M El Shahat,[2] Mohie E Ibrahim,[2] Ahmed A Salem.[2] *[1]OB/GYN, Yale University, New Haven, CT, USA; [2]OB/GYN, Benha School of Medicine, Benha, CT, USA.*

Objective: Growing evidence points to a role for endogenous opioids in the regulation of insulin secretion and carbohydrate metabolism in hyperandrogenemic women. This study assessed effects of long-term inhibition of the opioid system using naltrexone in clomiphene citrate (CC)-resistant women with PCOS.

Methods: Women with PCOS who failed to ovulate in response to CC (up to 200mg) were evaluated. All subjects received 50mg of naltrexone daily for 6 months. After 12 weeks, patients who did not ovulate on naltrexone alone also received CC (50mg/day for 5 days; the dose increased in 50mg increments up to 200mg). Ovulations were detected sonographically and by serum progesterone (on day 21). Evaluations performed at the baseline, at 12 weeks, and at the end of the study included: body mass index (BMI), hirsutism, acne, fasting insulin and glucose, total testosterone, LH and FSH.

Results: The enclosed table summarizes major outcomes. In addition, during naltrexone monotherapy 5 of 16 (31.2%) amenorrheic women began menstruating and 2 of 14 (14.2%) oligomenorrheic women resumed regular menses. After naltrexone+CC, 6 of 11 amenorrheic women began menstruating and 10 of 12 oligomenorrheic women resumed regular menses.

Conclusion: Naltrexone monotherapy improved menstrual cyclicity, hyperandrogenism, LH secretion and hyperinsulinemia in CC-resistant women with PCOS. Furthermore, naltrexone improved CC sensitivity, resulting in a significant number of pregnancies. We propose that naltrexone is effective in the treatment of clinical, endocrine and metabolic aspects of PCOS and, in particular, it improves sensitivity to CC.

	Baseline (N=30)	Naltexone (N=30)	Naltexone + CC (N=27)
BMI	35.6±2.2	31.4±1.1*	31.6±1.4*
No. of women who ovulated (%)	0/30 (0%)	3/30 (10%)	19/27 (70.3%)*†
No.of women who conceived	0/30 (0%)	0/30 (0%)	9/27 (33.3%)*†
Hirsutism score	8.2±0.7	6.7±0.5*	6.6±0.7*
Acne score	1.42±0.16	0.89±0.12*	0.9±0.13*
Fasting glucose to insulin ratio	3.48±0.27	4.36±0.5*	4.3±0.38*
Total testosterone	0.9±0.05	0.65±0.01*	0.62±0.06*
LH to FSH ratio	2.0±0.23	1.7±18*	1.5±0.28*

Values are presented as means ± SD; * significant vs. baseline (P<0.05); † significant vs. naltrexone monotherapy (P<0.05).

511

Inflammation and Endothelial Dysfunction in Women with Polycystic Ovary Syndrome. Mohamed I Mohamed,[1,2] Antoni J Duleba.[*1] *[1]OB/GYN, Yale University, New Haven, CT, USA; [2]OB/GYN, Benha School of Medicine, Benha, Egypt.*

Objectives: Polycystic ovary syndrome (PCOS) is associated with cardiovascular risk factors including insulin resistance, dyslipidemia, systemic inflammation and endothelial dysfunction. C-reactive protein (CRP) is a marker of systemic inflammation and an activator of endothelium. Urinary albumin excretion is a marker of endothelial dysfunction reflecting the integrity of glomerular endothelium. However, the relationship of CRP with urinary albumen and other cardiovascular risk factors remains unclear. This study was designed to evaluate CRP and urinary albumen in relation to clinical, endocrine and metabolic parameters in women with PCOS.

Materials and Methods: The study included 82 hyperandrogenic women with PCOS; subjects with diabetes, hypertension and non-classical congenital adrenal hyperplasia were excluded. Evaluations were performed 3-9 days after spontaneous or progestin-induced menses. Studied parameters included body mass index (BMI), blood pressure (BP), CRP, urinary albumin excretion, testosterone, 17-hydroxyprogesterone LH, FSH, lipid profile, vitamin E, as well as glucose and insulin during 2-hour glucose tolerance test. Measures of insulin sensitivity included quantitative insulin sensitivity check index (QUICKI) and insulin sensitivity index (ISI). Statistical evaluation consisted of linear regression analysis.

Results: Significant elevation of CRP (>3 mg/L) was observed in 50% of patients. In univariate analysis, CRP correlated most significantly with BMI (r=0.62; P<0.0001), diastolic BP (r=0.45; P<0.0001), ISI (r=-0.5; P<0.0001), and vitamin E (r=-0.34; P=0.005). In a multiple regression analysis, independent predictors were BMI (P=0.0005), diastolic BP (P=0.026) and ISI (P=0.03); R^2 of this model was 0.47. Microalbuminuria (urinary albumen 20-200mg/L) was detected in 23% of subjects. In univariate analysis, urinary albumen correlated with diastolic BP (r=0.24; P=0.05) and 17-hydroxyprogesterone (r=0.49; P<0.0001); there was no correlation with CRP or measures of insulin sensitivity. In multiple regression analysis, independent predictors of urinary albumen were diastolic BP (P=0.004) and 17-hydroxyprogesterone; R^2=0.33.

Conclusions: PCOS is associated with a high rate of significant systemic inflammation and endothelial dysfunction. It appears that CRP is an independent risk factor from microalbuminuria suggesting distinctly different mechanisms involved in regulating these markers of cardiovascular risks.

FRIDAY

512

Increased Secretion of Amylin in Women with Polycystic Ovary Syndrome. Summer James, Xin Ma, Manubai Nagamani.* *Division of Reproductive Endocrinology, OB/GYN, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: Most women with polycystic ovary syndrome (PCOS) are insulin resistant and are at high risk for type2 diabetes. Role of insulin in PCOS has been extensively studied. There are hormones other than insulin that play an important role in glucose homeostasis. Amylin is a 37 aminoacid peptide co secreted with insulin from the pancreatic ß cells. Receptors for amylin have been demonstrated in the ovaries.The role of amylin in women with PCOS has never been studied.

Methods: Twenty women with PCOS who had anovulatory cycles and evidence of hyperandrogenism were recruited for the study. Ten women with ovulatory cycles who matched the PCOS patients in BMI served as controls. After a high carbohydrate diet for 3 days, an oral glucose tolerance test (OGTT) was performed. Blood samples were obtained at fasting and 1, 2 and 3 hours for measurement of glucose, insulin and amylin. The area under the curve (AUC) for insulin, amylin and glucose was calculated. Levels of testosterone, DHEAS, FSH and LH were also measured in all patients. Eight women with PCOS were treated with metformin for 6 months. Amylin levels were measured before and after treatment.

Results: Fasting insulin levels (23.1 $\pm$ 2.4µU/ml vs 7.1 $\pm$ 10 µU/ml [$\pm$ SE]) and area under the curve (AUC) of insulin (476.5 $\pm$ 71.5 vs 156.3 $\pm$ 20.1µU/ml) during OGTT were significantly increased (P <0.001) in women with PCOS compared to control women, while the glucose levels were normal indicating insulin resistance. Fasting amylin (12.1 $\pm$ 2.4 vs 7.7 $\pm$ 0.7 pM) and amylin AUC (91.2 $\pm$ 10.8 vs 28.6 $\pm$ 5.4 pM) were also significantly (P < 0.001) increased in women with PCOS. There was significant positive correlation between insulin and amylin at fasting (r =0.64, P <0.02) and after glucose ingestion (r=.582, P <0.04). There was no correlation between basal glucose and amylin levels. However, amylin response (AUC) during OGTT positively correlated with the glucose responce (r =0.674, P <0.01). Metformin treatment resulted in significant decrease in amylin levels.

Conclusions: (1) there is hypersecretion of amylin in women with PCOS, (2) increased production of amylin in PCOS may be a consequence of co-regulation of insulin and amylin.(3) treatment with metformin reduces not only insulin but also amylin secretion, (4) role of amylin in the development of insulin resistance and ovarian dysfunction in women with PCOS needs further investigation.

513

Serum Anti-Mullerian Hormone (AMH) Levels Are Not Influenced by Insulin Infusion in Women with PCOS. Mickey S Coffler, Deborah S Wachs, Alice S Park, Ketan S Patel, Michael H Dahan,* Richard Y Yoo, Pam J Malcom, R Jeffrey Chang.* *Reproductive Medicine, UCSD, La Jolla, CA.*

Objective: Circulating AMH levels in women with PCOS are significantly higher compared to those of normal women. The basis for this difference has been attributed to increased follicle number in PCOS women as AMH mRNA expression is most prominent in preantral and small antral follicles. In addition, serum AMH has been shown to exhibit a tight positive correlation with small antral follicles of 2-5 mm size. Recent studies have demonstrated a direct correlation between fasting insulin levels, insulin resistance and levels of serum AMH which suggests that insulin may be related to increased AMH production. In vivo studies to determine whether insulin has an effect on AMH synthesis and secretion have not been performed. The aim of our study was to test the hypothesis that insulin increases AMH levels in PCOS.

Methods: 7 women with PCOS and 7 normal women were recruited for study. Each PCOS subject underwent a 10 hour hyperinsulinemic-euglycemic clamp at an insulin infusion rate of 200 mU/min. Glucose levels were clamped at 85 mg/dl. Levels of insulin and AMH were measured at baseline and at the end of the study. In both groups, baseline levels of reproductive hormones were determined. A two tail paired t-test was used to compare serum hormone and AMH levels.

Results: In PCOS women, the mean (±SE) age and BMI were 29.0 ± 2.3 and 40.1 ± 2.0, respectively, compared with that of normal women, 28.6 ± 1.5 and 27.4 ± 1.9. As expected, in PCOS subjects serum testosterone and androstenedione were significantly higher than those of the normal group. In addition, in PCOS serum LH was increased compared to normal women whereas no differences in FSH and serum estrogens were observed between groups. During insulin infusion, circulating insulin levels acutely increased from a mean of 49 ± 14 to 655 ± 19 µU/ml. These levels were kept constant until the end of the 10 hr infusion. Mean serum AMH prior to, 7.61 ± 1.13 ng/ml, and at the end of infusion, 7.85 ± 1.29 ng/ml, were not significantly different.

Conclusions: Notwithstanding the direct correlation between insulin and AMH levels found by some investigators, we were unable to demonstrate a direct effect of acute insulin infusion on serum AMH. These findings do not exclude the possibility that an effect of insulin on AMH production may require chronic exposure to hyperinsulinemia.

514

Granulosa Cells Isolated from PCOS Women Have Altered Steroid Biosynthesis and *CYP19* and *CYP11A1* Gene Expression in Long-Term Culture. Jessica K Wickenheisser,[1] Richard S Legro,*[1,2] Jan M McAllister.*[1,2] *[1]Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA, USA; [2]Obstetrics and Gynecology, The Pennsylvania State University College of Medicine, Hershey, PA, USA.*

Polycystic ovary syndrome (PCOS) is a common endocrinopathy characterized by hyperandrogenism and altered ovarian follicular development. In the PCOS ovary, overproduction of androgens by theca cells, and an apparent lack of aromatization to estrogens in granulosa cells (GCs), leads to a high androgen: estrogen ratio. In order to further understand the mechanisms regulating GC steroidogenesis in PCOS, we have begun to investigate steroid biosynthesis and steroidogenic enzyme gene expression in GCs in long-term culture.

Objective: To compare aromatase activity, progesterone (P4) biosynthesis, and mRNA abundance of cytochrome P450 cholesterol side-chain cleavage *(CYP11A1)* and cytochrome P450 aromatase *(CYP19)* in normal and PCOS GCs in long-term culture.

Methods: GCs were isolated from 5-8 mm follicles from normal or PCOS ovaries and propagated in culture for 31-38 population doublings. Following treatment with and without 10 µM forskolin (FSK), an activator of adenylate cyclase, aromatase activity was measured by tritiated water assay, P4 was measured by RIA, and CYP19 and CYP11A1 mRNA abundance were assayed by quantitative real-time PCR.

Results: As compared to GCs propagated from normal women, PCOS GCs were observed to have elevated aromatase activity, P4 production, and CYP19 and CYP11A1 mRNA abundance. FSK stimulated aromatase activity, P4 production, and CYP19 and CYP11A1 mRNA abundance in both normal and PCOS GCs. Transient transfection with CYP11A1 and CYP19 promoter constructs demonstrated an increased activity of these promoters in PCOS GCs, suggesting increased activation of these genes in PCOS GCs.

Conclusions: PCOS GCs in long-term culture are intrinsically different than normal GCs, with a phenotype of significantly elevated aromatase activity and P4 biosynthesis that correlates with increased mRNA abundance and transcription of the *CYP19* and *CYP11A1* genes. The observation of increased aromatase activity in PCOS GCs is in agreement with previous reports of PCOS GCs in primary culture. The ability of PCOS granulosa to express aromatase in culture makes them an effective resourse for examining the mechanisms involved in dysregulation of aromatase in the PCOS ovary.

515

Serum Anti-Mullerian Hormone Concentrations Are Not Altered by Acute Administration of Follicle Stimulating Hormone in Polycystic Ovary Syndrome and Normal Women. Deborah S Wachs, Mickey S Coffler, Pamela J Malcom, R Jeffrey Chang.* *Department of Reproductive Medicine, University of California, San Diego, San Diego, CA, USA.*

Objective: In the human ovary, expression of anti-mullerian hormone (AMH) is detected primarily in granulosa cells of preantral and small antral follicles. These findings are consistent with the reported tight correlation between circulating AMH levels and the number of small antral follicles (2-5 mm) in normal and PCOS women. In addition, the greater follicle count in PCOS is mirrored by significantly higher serum AMH levels compared to those of normal women. Despite the potential utility of AMH measurements in evaluating ovarian physiology and function, the regulation of AMH remains poorly understood. The objective of this study was to determine whether follicle stimulating hormone (FSH) acutely regulates granulosa cell AMH production in women with polycystic ovary syndrome (PCOS) and normal women.

Methods: A prospective study to compare ovarian responses in two groups of women was conducted at the GCRC in a tertiary academic medical center. Women with PCOS, 18-35 years (n = 16), and age-matched normal ovulatory controls (n = 11), were recruited for study. Serial blood samples were obtained over a 24 hour period following intravenous injection of recombinant human FSH, 150 IU. The main outcome measure was serum AMH responses following FSH administration.

Results: Basal serum AMH levels were markedly increased in women with PCOS compared to that observed in normal women. Following FSH injection, PCOS women failed to demonstrate changes in circulating AMH over 24 hr. A similar lack of alteration in serum AMH was observed in normal women.
Conclusions: Based on these findings we conclude that, in PCOS and normal women, acute administration of FSH does not effect granulosa cell AMH production.

516

Safety Profile of Rosiglitazone during the First Trimester of Pregnancy. Ghassan F Haddad, Cristiano Jodicke, Michael A Thomas,* Mira Aubuchon. *Obstetrics and Gynecology, Universtiy of Cincinnati Medical Center, Cincinnati, OH, USA.*
Objective: To examine the safety profile of rosiglitazone in the first trimester of pregnancy.
Introduction: Polycystic ovarian syndrome (PCOS) is a common reproductive disorder marked by insulin resistance. PCOS patients have an increased risk of miscarriage, gestational diabetes, macrosomia, caesarian deliveries, and pre-eclampsia. The incidence of these complications is decreased with the use of metformin, a pregnancy class B biguanide insulin sensitizer. However, the significant gastrointestinal disturbances associated with metformin often lead to its discontinuation. Rosiglitazone is a pregnancy class C thiazolidinedione insulin sensitizer with few side effects and is very effective at inducing ovulation in PCOS patients; however, there is paucity of data on its use during pregnancy.
Materials and methods: This case-series was compiled through an IRB-approved retrospective chart review of PCOS patients who have gotten pregnant at our center between January of 2003 through December of 2005. PCOS patients who refused metformin therapy due to side effects and who subsequently achieved pregnancy (with documented fetal heart motion) on rosiglitazone were included. After thorough counseling on the limited data regarding rosiglitazone use in pregnancy, 8 patients elected to continue rosiglitazone through the first 12 weeks of pregnancy. Pregnancy outcomes for those 8 patients were obtained by chart review or, if unavailable, through telephone contact with patients.
Results: Six of 8 patients delivered healthy babies at term; the remaining 2 had uncomplicated ongoing late third trimester pregnancies at the time of this submission. No congenital anomalies were noted. None of the pregnancies were complicated by diabetes, preterm delivery, or pre-eclampsia.

Table1: Demographics (mean ± SD)

Age (yrs)	Weight (lbs)	Gravidity	Parity	Mean rosiglitazone dose (mg)	# of weeks preconception used	# of weeks gestation used
28±2.8	203±63	1.3±1.4	0.4±0.5	5.5±2.1	22±17	11.9±1.6

Table 2: Mode of conception

	Spontaneous	Ovulation induction	Fresh IVF
Number (%)	3 (37.5%)	4 (50%)	1 (12.5%)

Summary: Rosiglitazone use was not associated with any maternal or fetal complications when taken during the first trimester. To our knowledge, our case series is the largest to describe the safety of rosiglitazone use in pregnancy.
Conclusion: Rosiglitazone taken pre and post-conception was not associated with adverse maternal or fetal outcomes.

517

Serum Aldosterone Levels in PCOS Women. O Muneyyirci-Delale,[1,3] N Dalloul,[1] I Joulak,[1] V Nacharaju,[1] L Yang,[1] H von Gizycki,[2] A Adejana.[1,3] (SPON: Pamela Stratton). *[1]Dept. of Obstetrics and Gynecology, SUNY Downstate Medical Center, Brooklyn, NY, USA; [2]Computing Science Center, SUNY Downstate Medical Center, Brooklyn, NY, USA; [3]Dept. of Obstetrics and Gynecology, Kings County Hospital Center, Brooklyn, NY, USA.*
Objective: Plasma aldosterone in women correlated directly with visceral adipose tissue and inversely with insulin sensitivity. Women with PCOS have hyperinsulinemia, selective insulin resistance and increased cardiovascular risk independent of obesity. PCOS patients are commonly treated with spironolactone and glucophage to lower androgen levels, improve hyperandrogenic manifestations and menstrual cycle. Dietary magnesium prevents fructose induced insulin sensitivity in rats and magnesium supplement reduces development of diabetes in rat model of spontaneous NIDDM. Hence we included treatment with magnesium oxide also. We earlier determined free fatty acid (FFA) levels in these groups and found that they were unchanged in glucophage and magnesium oxide treated groups but significantly decreased in spironolactone treated group. We wanted to investigate how aldosterone levels change in these group.
Material and Methods: After obtaining consent from patients, 36 women with PCOS were randomly divided into three groups. Patients in group 1 (n=14) were treated with 500 mg glucophage po bid, group 2 (n=10) were treated with 400 mg magnesium oxide po bid, and group 3 (n=12) were treated with 50 mg spironolactone po bid for 12 weeks. A glucose tolerance test with 75 g glucose load was performed before and after treatment, collecting blood at 0, 1 and 2 hours. Aldosterone levels were compared by repeated measure ANOVA.
Results: Pre-treatment aldosterone levels decreased in response to glucose load, whereas this decrease was not found after treatment in glucophage group. Aldosterone levels were expressed as mean ± SE. The aldosterone levels were fasting: 78.8 ± 15.2 pg/ml and one hour after glucose load: 57.99 ± 14.6 pg/ml (p=0.03). This decrease was not found after treatment with glucophage (p=0.59). The decrease in aldosterone in response to glucose was observed in spironolactone group at both pre (p=0.006) and post (0.045) treatment. Magnesium oxide group did not show this decrease at pre or post treatment. As expected the basal aldosterone levels increased significantly after treatment with spironolactone (<0.0001).
Conclusion: Aldosterone levels decreased in response to glucose load in PCO patients.

518

Enhanced Androgen Responses to Follicle Stimulating Hormone Stimulation in Women with Polycystic Ovary Syndrome Compared to Normal Women. Deborah S Wachs, Mickey S Coffler, Rodolfo Rey, Pamela J Malcom, R Jeffrey Chang.* *Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA, USA.*
Objective: In PCOS excess ovarian androgen production has been attributed to increased theca cell responsiveness to luteinizing hormone (LH) stimulation. However, it is also recognized that other factors may contribute to hyperandrogenemia. Relative to this notion, in vitro studies have demonstrated that granulosa cell stimulation may influence theca cell androgen responsiveness to LH. Clinical studies specifically designed to examine the role of the granulosa cell on ovarian androgen production have not been performed. To determine whether the granulosa cell may exert an influence on theca cell androgen production, androgen responses to follicle stimulating hormone (FSH) administration were assessed in women with polycystic ovary syndrome (PCOS) and normal women.
Methods: A prospective study to compare ovarian responses in two groups of women was conducted at the GCRC in a tertiary academic medical center. Women with PCOS, 18-35 years and normal ovulatory controls were recruited for study. Serial blood samples were obtained over a 24 hour period following intravenous injection of recombinant human FSH, 150 IU. The main outcome measures were serum androstenedione (A), testosterone (T), dehydroepiandrosterone (DHEA), and 17-hydroxyprogesterone (17-OHP) responses following FSH administration.
Results: Basal serum A, T, and 17-OHP levels were markedly increased in women with PCOS compared to that observed in normal women whereas serum DHEA levels were similar between groups. Following FSH injection, PCOS women exhibited increases in serum A, DHEA, and 17-OHP that were greater than corresponding increments observed in the control group. T levels remained relatively unchanged in both PCOS and normal women.
Conclusions: The findings show that FSH stimulates ovarian androgen production in PCOS and, to a lesser extent, normal women, which is consistent with in vitro studies. That the magnitude of androgen responsiveness was greater in PCOS women compared to normal women suggests a role for granulosa cell-mediated theca cell androgen production in this disorder.

519

Serum Anti-Mullerian Hormone (AMH) Concentrations in Oligomenorrheic Adolescents without Evidence of Hyperandrogenism and Girls with Polycystic Ovary Syndrome. Alice S Park, Deborah S Wachs, Mickey S Coffler, Richard Y Yoo, Pamela J Malcom, R Jeffrey Chang.* *Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA, USA.*
Introduction: Adolescent girls with polycystic ovary syndrome (PCOS) exhibit excessive hair growth and irregular menstrual bleeding much like those of adult women. In addition, it has been demonstrated that ovarian morphology is also similar in both groups. Specifically, ovarian volume and antral follicle number are significantly greater in PCOS individuals compared to normal ovulatory controls. Recently, it has been shown that serum AMH correlates tightly with antral follicle counts and may have clinical utility in the diagnosis

of PCOS. Based on this consideration and diagnostic criteria (Rotterdam) that PCOS may exist in women with oligomenorrhea and PCO, we undertook to assess serum AMH levels in non-hirsute, oligomenorrhea girls and adolescent girls with PCOS.
Objective: To determine whether serum AMH levels differ between oligomenorrheic adolescents without evidence of hyperandrogenism, adolescent PCOS girls, and normal ovulatory girls.
Methods: Serum AMH was measured in a total of 53 adolescent girls between the ages of 12-19. The groups comprised of oligomenorrhea (OLIGO) without evidence of hyperandrogenism (n=12), adolescent PCOS (n=26), and normal ovulatory girls (n=15).
Results: The median serum AMH level for OLIGO girls was 6.4 ± 2.3 ng/ml (95% CI: 4.7-8.1) higher than that of the normal adolescent group, 4.5 ± 2.4 ng/ml (95% CI: 3.0-6.0) whereas compared to adolescent PCOS girls, 6.6 ± 3.5 ng/ml (95% CI: 5.5-7.8), the values were equivalent. Analysis of variance failed to detect any statistically significant differences among groups.
Conclusions: The present study showed that AMH levels in OLIGO females were elevated compared to normal adolescent controls and similar to levels observed in adolescent PCOS girls. The lack of statistical differences among groups was likely due to the relatively small number of normal and OLIGO subjects studied. The findings suggest that in OLIGO girls AMH levels are elevated, which may reflect similar ovarian morphology as that of adolescent PCOS girls.

520

***CTF18* Plays an Essential Role in Mammalian Gametogenesis.** Karen M Berkowitz,*[1] Lydia R Koenig,[1] Fang Yang,[2] P Jeremy Wang,[2] Thomas A Jongens,[3] Klaus Kaestner.[4] *[1]Department of Obstetrics and Gynecology and Center for Research on Reproduction and Women's Health, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [2]Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA; [3]Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [4]Department of Genetics and Institute of Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.*
Objective: CTF18/Cutlet encodes an evolutionarily conserved protein that is crucial for germline development in the fly and that is essential for faithful transmission of chromosomes during DNA replication in yeast. The objective of our studies is to determine the function of CTF18 in mammalian germ cell development.
Methods: We generated a mouse model that lacks CTF18 by utilizing Cre/loxP technology to direct site-specific recombination. Initially we cloned and characterized the human and mouse orthologues of CTF18. Northern blot analysis was performed to reveal the expression pattern of CTF18 RNA in human and mouse tissues. Western blot and immunohistochemical analyses of mouse testis and ovary were performed to demonstrate expression of CTF18 protein. The phenotypic consequences of CTF18 deletion were assessed by gross, histological, chromosomal, and immunofluorescence examination of CTF18-null and wild-type gonads.
Results: Adult mutant male and female mouse gonads are much smaller and morphologically abnormal compared to those of their wild-type adult littermates. Seminiferous tubules from CTF18-mutant males show a paucity of spermatids and spermatozoa, and they contain large multi-nucleated cells that appear to be degenerating. Some areas of tubules are almost devoid of spermatogenic cells. Ovaries from CTF18-mutant females are smaller and contain fewer follicles than wild-type females. Many of the follicles are degenerating and contain misshapen oocytes. In addition, chromosomal spreads from CTF18-null mouse gonads reveal a clear defect during meiosis.
Conclusion: CTF18 plays a significant role in mammalian gametogenesis, and likely fertility.

521

Spastin Is a Novel Transcriptional Coactivator of HOXA10 in Endometrial Gene Regulation. Gaurang S Daftary,* Amy M Tetrault, Jennifer M Sarno, Hugh S Taylor.* *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objective: HOXA10 is a transcription factor required for uterine development and endometrial receptivity. Spastin is a cytoplasmic ATPase implicated in microtubule dynamics. Although, human spastin has a nuclear localization signal, so far it has no ascribed role in either the reproductive tract or in the nucleus in any tissue. Here we identify spastin as a novel cofactor in HOXA10 mediated endometrial gene transcription.
Methods: We conducted a screen to identify novel HOXA10 cofactors by immunoprecipitation of Ishikawa nuclear extract using anti-HOXA10 followed by sequencing. Spastin expression was determined in Ishikawa and endometrial stromal cells by RT-PCR. In Luciferase reporter assays, Ishikawa cells were transfected with either 4.0 µg spastin-myc chimera, 4.0 µg pcDNA/HOXA10 or both and empty pcDNA as a control. Cells were additionally transfected with a previously characterized HOXA10 responsive EMX2-luciferase vector. Transfections were done in triplicate. Luciferase activity was assayed 48 hours post-transfection. Electrophoretic Gel Mobility Shift Assay (EMSA) was performed using ^{32}P-labeled EMX2 HOXA10 responsive element and Ishikawa nuclear extract after transfection with 4.0 µg spastin-myc.
Results: Spastin expression was identified in endometrial stromal and Ishikawa cells. Spastin co-immunoprecipitated with HOXA10 using nuclear extracts from these cells. In EMX2-Luciferase assays, reporter activity decreased with HOXA10 transfection ($p<0.004$) whereas it increased with spastin ($p<0.02$) transfection. Additionally, spastin reverses HOXA10 mediated repression of EMX2-Luciferase activity. In EMSA, the shift obtained with Ishikawa nuclear extract was supershifted using either anti-HOXA10 or anti-myc, indicating that HOXA10 and spastin directly interact.
Conclusion: Here we demonstrate that Spastin, an ATPase mutated in hereditary spastic paraplegia, is a novel cofactor in HOXA10 mediated endometrial transcriptional regulation. Spastin is expressed in endometrial epithelial and stromal cells where it co-localizes with HOXA10. Additionally, Spastin directly binds an EMX2 regulatory element in conjunction with HOXA10, thereby altering target gene expression. We identify a novel role for Spastin as a HOXA10 cofactor in the regulation of uterine gene expression. It is likely that Spastin functions widely as a HOX cofactor in non-reproductive tissues.

522

Expression, Regulation, and Possible Function of the Alternative Estrogen Receptor, GPR30, in Human Endometrial Cells. Steven L Young,*[1] Jessica G Scotchie,[1] Wilder A Palomino,[2] Bruce A Lessey.*[2] *[1]Obstetrics & Gynecology, University of North Carolina, Chapel Hill, NC, USA; [2]Obstetrics & Gynecology, Greenville Hospital System, Greenville, SC, USA.*
Estrogen plays a critical role in endometrial function. Recently, GPR30, an integral membrane protein which can act via the EGF receptor, was shown to function as an estrogen receptor (Revankar, Science 2005). Expression of GPR30 has not been described in the uterus of any species, but a report suggests that GPR30 may mediate proliferative effects of estradiol and tamoxifen in endometrial cancer cells (Vivacqua, Mol Endocrinol 2006).
Objective: Describe the expression, regulation, and function of GPR30 in human endometrial cells.
Methods: Proliferative and secretory endometrium from healthy, normally cycling volunteers was obtained under an IRB-approved protocol. Immunolocalization and Western blot analysis of GPR30 was performed using a rabbit polyclonal antibody (Abcam, ab12563). Real-time RT-PCR for GPR30, Cyr61 and constitutive genes, PPIA and GUS utilized predesigned primers & probes (Applied Biosystems).
Results: Immunohistochemistry demonstrated endometrial GPR30 expression, predominantly in epithelial and endothelial cells. Staining was markedly darker in midsecretory specimens, but was non-uniform, so that only some cells in each gland were stained. Real-time qRT-PCR demonstrated GPR30 mRNA expression in both proliferative and secretory endometrium as well as ECC1, RL95-2, and HES cell lines. A small amount of transcript was seen in Ishikawa cells and none in Hec-1B cells. A candidate marker of GPR30 action, Cyr61 was stimulated 3-6 fold by treatment with 1-100 nM estradiol, diethylstilbesterol, 1µM progesterone or 10 µg/mL epidermal growth factor (EGF) in ECC1 and HES cells. Estradiol stimulation of Cyr61 mRNA was maximal at 1 nM and both EGF and estradiol effects were apparent 15 minutes after exposure. Changes in protein expression were apparent at 4 hours after exposure. Estrogen stimulation of Cyr61 was blocked by pretreatment with the anti-EGF receptor antibody, C225.
Conclusions: GPR30 mRNA and protein are expressed by human endometrium. Protein expression appears increased in the midsecretory phase, and is localized to specific epithelial cells, suggesting functional differences between endometrial epithelial cells in the same gland. A candidate marker of GPR30 action, Cyr61, is rapidly induced by physiologically relevant estradiol levels in an EGF receptor-dependent fashion.

523

The Forkhead Family of Transcription Factors Are Differentially Expressed and Regulated in Leiomyoma and Myometrial Cells. Anna V Hoekstra, Erin C Ward, Xhenxiao Lu, Jennifer L Hardt, Erica Marsh, Ping Yin, Serdar Bulun,* J Julie Kim.* *Division of Reproductive Biology Research, Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Objective: The forkhead family of transcription factors are involved in regulating cell proliferation, differentiation, oxidative stress and apoptosis. FOXO1 is differentially expressed in the endometrium and plays an important role during differentiation of the endometrium. In this study we investigated the expression and regulation of FOXO1, FOXO3 and FOXO4 in myometrial and leiomyoma cells to determine whether these proteins are differentially expressed and regulated in these two cell types and whether this may influence cell proliferation.

Methods: Cells were isolated from myometrial and leiomymoma tissues and grown in culture. Western blot analysis was used to evaluate expression of FOXO1, FOXO3 and FOXO4 proteins. Cells were also treated for 24 hours with various regulators of FOXO expression, including TNF-α, R5020 (progestin), 17-β estradiol, an Akt inhibitor, and dbcAMP. Western blots and proliferation assays were performed to assess the effect of these treatments on cell proliferation and expression of the FOXO1, FOXO3 and FOXO4.

Results: Leiomyoma cells showed a significantly lower protein expression of both FOXO1 and FOXO3 compared to myometrial cells. There was no difference in expression of FOXO4, however, between the two cell types. In myometrial cells, Akt inhibitor, at 6uM and 12uM concentrations, increased the expression of FOXO1. Treatments with 1uM R5020 increased the expression of FOXO1 and FOXO3. In response to 0.5mM dbcAMP, expression of FOXO1 and FOXO3 increased. TNF-α increased expression of FOXO3. Leiomyoma cells responded differently in that only Akt inhibition increased FOXO1 and FOXO3, while R5020 increased FOXO3 expression only. Cell proliferation assays revealed increased proliferation in leiomyoma cells when treated with estrogen or progesterone. TNF-α and Akt inhibitors significantly inhibited proliferation in both myometrial and leiomyoma cells.

Conclusions: FOXO1 and FOXO3 are differentially expressed and regulated in myometrial and leiomyoma cells. Since forkhead proteins negatively regulate proliferation and are pro-apoptotic, restoration of expression FOXO1 in leiomyoma through inhibition of the Akt pathway may represent a new therapeutic modality.

524

Decreased Oocyte-Granulosa Cell Communication in Diabetic Mice. Ann M Ratchford, Cybill R Esguerra, Kelle H Moley.* *OB/GYN, Washington University School of Medicine, St. Louis, MO, USA.*

In women, Type 1 or Type 2 diabetes has been found to negatively affect pregnancy by causing poor prenatal outcomes such as increased risk of congenital anomalies and early miscarriage. Maternal diabetes in murine models adversely affects pregnancy outcomes as well, with defects observed as early as the preovulatory oocyte stage of development. These defects include impaired meiotic maturation, abnormal metabolism, and increased apoptosis. During the early stages of oocyte development, gap junction communication between the oocyte and surrounding cumulus cells is critical for the regulation of oocyte growth and meiotic maturation. OBJECTIVE: To analyze the effect of maternal diabetes on gap junction communication between the oocyte and cumulus cells, and to investigate the expression levels of gap junction proteins, known as connexins (Cxs). METHODS: Cumulus cell enclosed oocytes (CEOs) were collected from diabetic and nondiabetic C57BL/6JXSL/J mice for use in FRAP (Fluorescence Recovery After Photobleaching) analyses to examine gap junction communication. The differential expression of connexin proteins was studied using real-time PCR, western blot analyses, and immunohistochemistry. RESULTS: FRAP analyses showed a 20% decrease in communication in diabetic CEOs (13.6 ± 4.1 % FRAP, n = 3 experiments) as compared to nondiabetic (34 ± 2.6 % FRAP, n = 3 experiments). Real time PCR confirmed the presence of Cx57 and Cx37, and revealed the presence of Cx26 mRNA in nondiabetic mouse oocytes. Cx57 and Cx26 mRNA was expressed more highly in the diabetic oocytes as compared to the nondiabetic oocytes, and more highly expressed in oocytes versus the surrounding cumulus cells. In contrast to the mRNA expression, immunohistochemistry and western analyses showed a decrease in Cx26 protein expression in diabetic oocytes as compared to nondiabetic. CONCLUSIONS: The decrease in Cx26 protein expression may lead to the decreased communication between the diabetic oocytes and surrounding granulosa cells. This may in turn cause the impaired meiotic maturation and poor pregnancy outcomes in diabetic mice. This work is funded by the National Institute of Child Health and Human Development Cooperative Program on Female Health and Egg Quality, U01 HD044691.

525

TGFβ Mediates Male-Female Seminal Fluid Signaling in the Human Cervix. Sarah A Robertson, David J Sharkey. (SPON: Joan S Hunt). *Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia.*

Objective: After intercourse, seminal fluid interacts with cervical cells to induce inflammatory changes that potentially influence female tract immune responses to seminal antigens and sexually transmitted pathogens. Soluble factors of unknown identity in seminal plasma activate expression of several pro-inflammatory genes including cytokines GM-CSF, IL-6, IL-8 and MCP-1. Our animal studies have identified TGFβ as a key signaling agent in semen, and all three isoforms of TGFβ are abundant in human seminal plasma. The purpose of this study was to investigate whether TGFβ1, TGFβ2, and TGFβ3 are mediators of seminal plasma signaling in the cervical inflammatory response of women. We also evaluated the interaction between TGFβ and IFNγ, a cytokine that inhibits TGFβ actions in mouse reproductive epithelia.

Methods: Primary cervical epithelial cells from ectocervix of hysterectomy tissues, or immortalised Ect1 ectocervical epithelial cells, were incubated with recombinant human TGFβ (isoforms 1, 2 or 3) +/- IFNγ, or with pooled human seminal plasma. Epithelial cell mRNA expression profile was evaluated by Affymetrix HG-U133Plus2 microarray and by QRT-PCR, and culture supernatants were analysed by ELISA to quantify GM-CSF, IL-6, IL-8 and MCP-1.

Results: Microarray data showed that Ect1 cell incubation with TGFβ3 induced expression of several, but not all the pro-inflammatory cytokine genes elicited by pooled seminal plasma. TGFβ1, TGFβ2 and TGFβ3 showed comparable capacity to stimulate >5-fold increases in both GM-CSF and IL-6 mRNA, and secretion of immunoactive GM-CSF and IL-6, in a dose-responsive manner. Addition of neutralising antibodies for individual TGFβ isoforms inhibited seminal plasma-induced cytokine synthesis. In contrast, TGFβ did not stimulate IL-8 or MCP-1 synthesis. IFNγ potently inhibited TGFβ-induced GM-CSF release and also inhibited seminal plasma-induced IL-8, but did not affect IL-6 secretion.

Conclusion: The data show that all three TGFβ isoforms are major active male-female signaling agents in human seminal plasma, while seminal IFNγ can inhibit TGFβ signaling to differentially attenuate cervical cytokine expression. Through their control of cervical cytokine expression profile, these seminal fluid factors are implicated in programming the phenotypes of local leukocyte populations with immune-regulatory actions, thereby influencing female reproductive tract immune competence for pregnancy and defence against sexually transmitted disease.

526

COUP-TFI Inhibition of SF-1-Induced Rat LHβ Promoter Activity Does Not Require Competitive Binding to the Same cis-Acting Element. Weiming Zheng, Lisa M Halvorson.* *OB/GYN, UT Southwestern Medical Center at Dallas, Dallas, TX, USA.*

Objectives: Chicken ovalbumin upstream promoter transcription factors (COUP-TFs; NRF2) are orphan members of the nuclear hormone receptor superfamily. COUP-TFI and COUP-TFII have been identified in an array of mammalian tissues. It has been reported that haploinsufficiency of COUP-TFII results in female reproductive dysfunction. Luteinizing hormone β-subunit (LHβ) gene expression is known to be dependent on stimulation by steroidogenic factor-1 (SF-1; NR5A1), acting through direct interaction with two cis-elements, termed GSEs. The objective of this study was to determine the effects of COUP-TFI/II on rat LHβ promoter activity.

Methods: Western blot was used to detect the presence of COUP-TFI/II protein. CV-1 cells were transfected with a rat -207/+5 LHβ promoter-reporter vector as well as expression vectors for COUP-TFI/II, and/or SF-1. Electrophoretic mobility shift assay (EMSA) was performed to identify COUP-TF binding sites in the rat LHβ promoter sequence.

Results: Both COUP-TFI and COUP-TFII protein were expressed in mouse primary pituitary cells and in the gonadotrope cell lines, LβT2 and αT3-1. Both COUP-TFs increased rLHβ promoter activity 7-fold when transfected individually. When cotransfected with SF-1, COUP-TFI and COUP-TFII significantly inhibited the stimulatory effects of SF-1 on rLHβ promoter activity, with greater inhibition by COUP-TFI. Mutation of the 3'-GSE cis-element, but not the 5'-GSE, eliminated the COUP-TF effect. Consistent with the transfection data, i*n vitro* translated COUP-TFI and COUP-TFII specifically bound to a probe spanning the 3'-GSE site, but not to the 5'-GSE site. A mutant COUP-TFI (C_{141}-s hCOUP-TFI) containing a mutation in the DNA-binding domain was unable to bind to the 3'-GSE probe. This mutant had no stimulatory effect on rLHβ promoter activity, but retained the ability

FRIDAY

to blunt SF-1 effects. Scanning mutations in the 3'-GSE probe were tested for their ability to bind the COUP-TFs and SF-1. The binding pattern was identical for the COUP-TFs, but differed from SF-1.
Conclusions: These observations indicate that the COUP-TFs transcriptionally activate the rat LHβ promoter through interaction with the previously identified 3'-GSE region. Based on analysis of the mutated COUP-TFI, repression of SF-1-mediated increases in rat LHβ promoter activity occurs through a mechanism other than competitive binding by these two transcription factors to the same cis-acting element.

527

HOXA10 Regulation of the Calcium Binding Protein S100A14 in Endometrium. Erin F Wolff, Hongling Du, Ivan Pena, Hugh S Taylor.* *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objectives: S100A14 is a member of the family of proteins which bind calcium through EF-hand Ca^{2+} binding motifs and are thought to be involved in signal transduction. S100A14 expression has been reported in several tissues and cancers of epithelial origin, including the uterus and ovary. Expression of S100A14 has not been reported in normal endometrium. S100A14 was identified in a mouse microarray as a target gene which is reciprocally regulated by HOXA10. HOXA10 is a transcription factor necessary for endometrial differentiation and receptivity. In this study we characterize S100A14 expression and regulation in murine and human endometrial cells.
Methods: Decidualized endometrium from reproductive aged women in the first trimester was obtained by dilatation and curettage under an approved HIC protocol. To determine if S100A14 was regulated by HOXA10, HESC, decidual, and Ishikawa cells were transfected at 60% confluence in triplicate with 4 ug pcDNA/HOXA10; transfection with the empty pcDNA served as a control. 48 hours after the transfection was complete (72 hours total), cells were trypsinized and RNA was extracted. cDNA was generated and qRT-PCR was performed in duplicate (N=4). Quantitative Real Time RT-PCR (qRT-PCR) was used to measure S100A14 expression in the endometrium, the human endometrial stromal cell line (HESC), first trimester human decidual cells, and in Ishikawa cells.
Results: S100A14 was expressed in murine and human endometrium. S100A14 was also expressed in Ishikawa, HESC, and first trimester human decidual cells. In Ishikawa cells, S100A14 was upregulated by 50% in cells transfected with HOXA10 relative to controls. Expression of S100A14 was decreased in decidua cells by 40% by HOXA10 transfection. In contrast, S100A14 expression in HESC was decreased by 70% in response to HOXA10 transfection.(p=0.014) S100A14 was expressed in endometrial epithelium where it is regulated by HOXA10.
Conclusions: S100 proteins were expressed in the endometrial epithelium. HOXA10 is required for endometrial receptivity; it functions as a transcription factor and is required for the regulation of multiple genes involved in embryo implantation. The expression of S100A14 was regulated by HOXA10 in endometrial epithelial cells. The tissue-specific and regulated expression of S100A14 suggests a role in endometrial receptivity.

528

In Preterm Fetal Sheep Systemic Low Dose Endotoxin Results in Moderate Brain White Matter Injury and Cytoarchitectural Alterations in the Cerebral Cortex. Markus Gantert,[1] Pawel Kreczmanski,[2] Nina Peters,[2] Christoph Schmitz,[2] Yves Garnier.*[1] *[1]Department of Obstetrics and Gynecology, University Hospital of Cologne, Cologne, Germany; [2]Department of Psychiatry and Neuropsychology, University Hospital of Maastricht, Maastricht, Netherlands.*
Objective: Clinical and epidemiological studies indicate that intrauterine infection increases the risk of perinatal brain damage especially in preterm fetuses. Moreover, it has been shown that both viral and bacterial perinatal infections increase the risk to develop neuropsychiatric disorders durinmg later life. In these cases specific cytoarchitectural alterations in the cerebral cortex have been found.
Methods: Fifteen fetal sheep were chronically catheterized at a mean gestational age of 107 ± 1 days (term is 147 days). Three days after surgery the fetuses received either 100 nanogram endotoxin (lipopolysaccharide, LPS derived from E. coli; O127:B8, Sigma-Aldrich) (n=9) or 2 ml 0,9% saline (n=6) i.v. Fetal sheep were monitored for seven consecutive days. Thereafter in vivo perfusion fixation with was performed. Immunohistochemical detection of glial fibrillary acidic protein (GFAP), calbindin, calretinin and parvalbumin was performed according to standard procedures described in the literature.
Results: (i) In control brains astrocytes showed a strong GFAP signal in both, cell bodies and processes. This was found in all cortical layers. After LPS administration astrocytes showed a decrease in GFAP immunoreactivity. (ii) In control brains, calretinin (CR) positive neurons were present in cortical layers I-III. The expression in both cytoplasm and processes was equally intensive. Neurons expressing CR were uni-, bi-, and multipolar. In the LPS treated brains, CR expression was significantly decreased in small and medium sized neurons. (iii) In comparison to CR expression, a positive reaction with calbindin (CB) was observed in a smaller number of neurons in the control brains. CB reactivity was mainly present in small and medium sized neurons of both cortex and caudate nucleus. (iv) In the control brain, extremely strong parvalbumin (PV) expression was observed in cortical layer III and in the striatum. After LPS the number of PV positive neurons decreased, especially in the cortical layer III.
Conclusion: Low dose endotoxin results in a mild systemic inflammatory response causing specific cytoarchitectural alterations in the cerebral cortex in preterm fetal sheep.

529

Hypothalamic NPY Expression Is Inversely Correlated with Plasma Leptin Concentrations in the Late Gestation Fetal Sheep. BS Muhlhausler,[1,2] JD Dorosz,[2] CL Adam,[3] PA Findlay,[3] IC McMillen.[1,2] (SPON: David M Olson). *[1]Sansom Research Institute, University of South Australia, Australia; [2]School of Molecular and Biomedical Science, The University of Adelaide, Australia; [3]Obesity and Metabolic Health Division, Rowett Research Institute, Aberdeen, United Kingdom.*
Objective: Previous studies have shown that the neuropeptides which make up the central neural network for the regulation of appetite are expressed before birth in the human and sheep. It is not know, however whether this central neural network can respond to signals of nutritional status before birth. The aim of the present study was to determine the effect of intravenous leptin infusion on the expression of central appetite regulating neuropeptides in the hypothalamus of the late gestation fetal sheep.
Methods: From 130 -134 d gestation (term = 150 ± 3d gestation) fetuses were infused with either recombinant ovine leptin (60μg/0.16ml/h, n=7) or saline (0.16ml/h, n=6). Whole brains were collected at postmortem and the expression of Neuropeptide Y (NPY, appetite-stimulation), Proopiomelanocortin (POMC, appetite-inhibition) and the leptin receptor (OBRb) were determined using *in situ* hybridization.
Results: Plasma leptin concentrations were significantly higher in leptin infused compared to saline infused fetuses both across the whole infusion period ($P<0.05$) and on day of postmortem (5.07 ± 0.79 vs 2.0 ng/ml ± 0.54 ng/ml, $P<0.02$). Total brain weight, but not fetal body weight, was higher in the leptin infused group (56.5 ± 1.1 vs 52.3 ± 1.3 g, $P<0.05$). There was no effect of leptin infusion on the expression of NPY mRNA in the fetal brain. There was, however, a significant inverse relationship between NPY mRNA expression and mean plasma leptin concentrations on the day of postmortem (NPY = -0.5 leptin + 0.69, $r^2=0.36$, $P=0.05$, n=11). There was no effect of leptin infusion on the expression of POMC or OBRb mRNA in the fetal brain.
Conclusions: These findings provide the first evidence that leptin may have the capacity to regulate the central expression of the appetite stimulating neuropeptide, NPY, before birth, and suggest that the central neural network for appetite regulation has the capacity to respond to peripheral signals of nutritional status before birth. These findings have clear implications for understanding how the central neural network for appetite regulation responds and adapts to altered levels of prenatal nutrition.

530

Maternal Caffeine Administration Decreases Cerebral Oxygenation in Near-Term Fetal Sheep. Stephen J Lee, Jonathon M Ross, Jordan A Lang, Takuji Tomimatsu, Jorge Pereyra Pena, Lawrence D Longo.* *Center for Perinatal Biology, Departments of Physiology and Obstetrics & Gynecology, School of Medicine, Loma Linda University, Loma Linda, CA, USA.*
Objectives: Caffeine, a known non-selective adenosine antagonist, has long been consumed world-wide, even during pregnancy, and it readily crosses both the placental and blood-brain barriers. Adenosine is a potent vasodilator of cerebral arteries, and also a potent suppressor of neuronal activity. Surprisingly, little research has examined the effects of this adenosine antagonist on fetal cerebral blood flow and cerebral oxygenation. Thus, we tested the hypothesis that adenosine inhibition by caffeine would significantly alter fetal cerebral oxygenation.

Methods: By use of a fluorescent O_2 probe with a laser Doppler flowmeter in the cerebral cortex, and the placement of sagittal sinus catheter, in 6 near-term fetal sheep we measured values of cortical tissue O_2 tension (tPO_2), sagittal sinus oxyhemoglobin saturation ([HbO_2]), and laser Doppler cerebral blood flow (LD-CBF) in response to 30 minutes of i.v. infusion of caffeine citrate (400 mg: 2 to 3 cups of coffee) into the near-term pregnant ewe.
Results: In response to maternal caffeine infusion, we observed rapid and significant decreases in fetal cortical tPO_2 (from 10±1 to 5±1 Torr), and sagittal sinus [HbO_2] (from 45 to 35%) ($p<0.01$ for each), accompanied by a modest 15% decrease in fetal LD-CBF. Fetal systemic arterial blood gas values did not change significantly.
Conclusions: Maternal caffeine administration significantly decreased fetal cerebral oxygenation, without affecting overall systemic oxygenation. These findings may have clinical relevance for the pregnant mother and fetus. (Supported by USPIHS, HD 03807).

531

Prostaglandin-Mediated Fetal Vascular Programming: Genetic and Prolonged Pharmacological COX Inhibition Impairs Responsiveness of the Ductus Arteriosus. Jeff Reese,* Naoko Brown, Stan D Poole, Patrick W O'Mara. *Dept. of Pediatrics, Vanderbilt University, Nashville, TN, USA.*
Background: Cyclooxygenase (COX)-derived prostaglandins relax the ductus arteriosus (DA) *in utero* and their withdrawal facilitates its closure. Although exposure to COX inhibitors during pregnancy can result in fetal DA constriction, some newborns paradoxically develop persistent patency of the DA (PDA). In mice, we previously showed that combined deletion of COX-1 and COX-2 results in PDA. Prolonged COX inhibition on d15-19 of gestation also leads to PDA, suggesting that prostaglandins may regulate DA development in addition to their role as vasoactive mediators.
Objective: We hypothesized that postnatal DA responsiveness is dependent on signaling via specific prostaglandin receptors which direct DA development in mid- to late-gestation.
Methods: Wild type (WT) fetal DA segments were collected on d14-19 (term=d19) and postpartum (n=10-18 DAs/timepoint, x3). Quantitative RT-PCR was performed for PGE receptors EP1-4. EP localization was examined by *in situ* hybridization. WT females were treated with selective COX-1 and COX-2 inhibitors on d15-19. Responsiveness of WT (n= 7), COX-1+COX-2 inhibited (n=9), and COX-1/COX-2 double null (n=5) DAs at term was examined in a cannulated microvessel myography system.
Results: EP2 expression was at the limits of detection; EP1 and EP3 increased slightly with advancing gestation, whereas EP4 increased on d15 and remained elevated on d16-19 of gestation. EP4 was localized to the DA smooth muscle and endothelium. At physiologic pressures, COX-inhibited and COX-deleted DAs had significantly greater resting dimensions than WT, but similar KCl-induced constriction (69, 67, 72% of baseline, respectively). Compared to WT, COX-inhibited and COX-deleted DAs had diminished response to indomethacin (18% vs 13, 6%, respectively) and variable response to L-NAME (9% vs 3, 26%, respectively). U46619 potently constricted all DAs. COX-inhibited and COX-deleted DAs had less oxygen-induced constriction than WT.
Conclusions: Disruption of the COX pathway *in utero* impairs postnatal DA closure due to failure of EP4-mediated signaling during a critical window of DA development. DAs from an environment of reduced or absent COX stimuli have less tone and less response to indomethacin but are responsive to oxygen and may have enhanced response to nitric oxide inhibition. These data suggest that prostaglandins play a novel role mediating DA development.

532

Effects of Clinical Doses of Betamethasone (BM) Used To Enhance Fetal Lung Maturation on Cerebral Blood Flow (CBF) in Fetal Sheep. Bettina Reinhardt,[1] Turhan Coksaygan,[2] Thomas Muller,[3] Harald Schubert,[3] Peter W Nathanielsz,*[2] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller University, Jena, Germany; [2]Center for Pregnancy and Newborn Research, Univ. of Texas, San Antonio, TX, USA; [3]Inst. of Lab Animal Sciences, Friedrich Schiller University, Jena, Germany.*
Direct i.v. infusion to the sheep fetus increases cerebovascular resistance resulting in decreased CBF independent of maturation of the fetal pituitary adrenal (HPA) axis and the cerebrovascular system (J Physiol 2000;528:619; J Physiol 2005;564:575).
Objective: To examine if similar effects occur after maternal BM administration at the dose used clinically to enhance fetal lung maturation.
Methods: Using fluorescent microspheres, we measured CBF in 11 brain regions in chronically instrumented fetal sheep at baseline and 3-5 and 24 h after two maternal i.m. injections of 110µg/kg BM at 0.73 gestation (n=7) or 170µg/kg at 0.87 gestation (n=6) corresponding to 8 or 12mg BM administered to a 70kg pregnant woman at 29 and 34 weeks gestation. These developmental stages are before and during the preparturient increase in HPA activity and maturation of the cerebrovascular system. Controls received an equal volume saline (n=6 or n=7, respectively). To determine BM effects in relation to sleep states, CBF was measured in additional animals at 0.87 gestation 24h after the first and 3-5h after the second injection of BM (n=10) or saline (n=7) in REM and NREM sleep.
Results: BM did not affect CBF in any brain region at either age when fetal sleep states were not considered. Considering the sleep states, BM decreased CBF in REM ($p<0.05$) and in NREM sleep ($p<0.01$) 3-5 h but not 24 h after injection by 35 to 40 % in most brain regions. The BM effect corresponds to fetal peak BM plasma concentrations.
Conclusion: BM at clinical doses reduces CBF transiently at 0.87 but not 0.73 gestation when the HPA axis and the systems regulating cerebrovascular tone are immature.

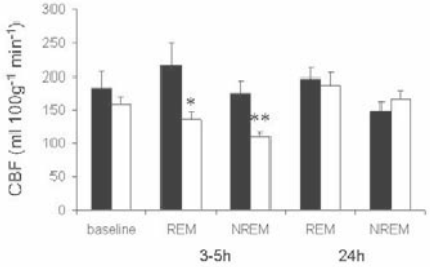


Fig. 1: Total CBF in saline (black) and BM treated fetuses (white) at 0.87 gestation in relation to sleep states. Mean±SEM, **p<0.01, *p<0.05 compared to controls.

533

A Possible Involvement of Local Cardiac Renin-Angiotensin System in Developmental Origins of Cardiovascular Disease. Hiroaki Itoh,*[1] Makoto Kawamura,[1] Shigeo Yura,[1] Haruta Mogami,[1] Tsuyoshi Fujii,[1] Norimasa Sagawa,[2] Shingo Fujii.[1] *[1]Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Department of Gynecology and Obstetrics, Mie University Graduate School of Medicine, Tsu, Mie, Japan.*
Background- Evidence has emerged that fetal undernutrition is a risk factor for cardiovascular disorders in adulthood. Recently, the local expression of angiotensinogen and related bioactive substances has been demonstrated to augment cardiac remodeling, i.e. fibrosis and hypertrophy, which is called as local cardiac rennin-angiotensin system (RAS). The aim of the present study was to elucidate the possible involvement of the local cardiac RAS in fetal undernutrition-induced cardiovascular disorders. ***Materials and Methods-*** Using a mouse animal model of fetal undernutrition by maternal food restriction (30% restriction 10.5-18.5 dpc; UN offspring), we assessed the cardiac remodeling, as a risk factor of cardiovascular disease, by measuring cardiomegaly (cardiac weight [mg]/body weight [g]), cardiomyocyte enlargement (mean transverse diameter), coronary perivascular fibrosis (digital image analysis after Sirius Red Staining) in the UN and normal (NN) offspring at 8 and 16 wks. The whole cardiac tissue were collected at 18.5 dpc and at 3, 8 and 16 wks, in which mRNA expression of angiotensinogen (Ang), ACE, ANP, BNP, and endothelin-1 (ET-1) was measure by quantitative RT-PCR. The Immunohistochemistry of angiotensin II and ET-1 was carried out. ***Results-*** Significant augmentation was observed in perivascular fibrosis of the coronary artery (16 wks, $P<0.05$), cardiomegaly (5.79 ± 0.78 [n=14, mg/g] v.s. 5.05 ± 0.18 [n=16, mg/g] at 16 wks, $P<0.01$) and cardiomyocyte enlargement (16.6 ± 0.3 [n=10, maicrometer] v.s. 14.3 ± 0.3 [n=10, micrometer] at 16 wks, $P<0.01$), concomitant with a significant augmentation of Ang ($P<0.05$) and ET-1 ($P<0.01$) mRNA expression in UN offspring at 16 wks, as compared with NN offspring. There occurred a tendency to increase in immunostaining for angiotensin II as well as ET-1 in UN offspring at 16 wks, compared to NN offspring. In 18.5 postcoitum days, undernutrition in utero significantly elevated Ang, ACE, and ET-1 mRNA levels in the fetal heart ($P<0.05$ for all). ***Conclusions-*** It was suggested that fetal undernutrition activated the local cardiac RAS, which contributed, at least partly, to the development of cardiac remodeling in later life.

FRIDAY

534

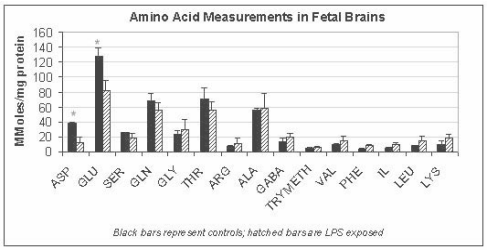
Early Detection of Vascular Changes in Fetal Haemodinamic Using a Novel Global Acquisition & Signal Processing (G.A.S.P.) Software for the Multigate Spectral Doppler Analysis in Fetal Imaging: A Twin Study.
Gabriele Urban,[1] Pasquale Patrizio,[2] Fabio Sanguineti,[2] Stefano Ricci,[3] Piero Tortoli,[3] Patrizia Vergani, Charles J Lockwood,[*] Michael J Paidas. *[1]Obstetrics and Gynecology, University of Milan Bicocca, Monza, Italy; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [3]Electronics and Telecommunication, University of Florence, Florence, Italy.*
Objective: To study materno-fetal hemodynamic in Bi/Bi twin pregnancy. In this paper we propose a new US method, named Multigate Spectral Doppler Analysis (MSDA), that overcomes the limitation related to the use of a single sample volume. In this method, 128 small sample volumes are aligned along an US scan line that intercepts the vessel. The Doppler data from each sample volume can be independently analysed to produce a high resolution flow profile.
Methods: The Multigate Spectral Doppler Analysis (MSDA) system employed in this study consisted of commercial ultrasound machine (Aloka SSD1400) connected to personal computer (PC) where a proprietary electronic board was plugged and where the G.A.S.P software ran. Imaging was performed within standard safety guidelines. We scanned left and right uterine arteries and fetal aorta in 7 Bi/Bi twin gestation at 6 week 9 week and at 11 week of gestation after in-vitro fertilization. We evaluate velocities profiles, wall distension rate and shear rate calculating from consecutives cardiac cycles.
Results: We were successfully able to generate a velocity profiles from all the vessels interrogated maternal and fetal. No significant differences were found between twin A and twin B of velocity profiles, WSR and rWDR.
Conclusions: We found a substantial difference between conventional Doppler results and velocity profile from MGSD. Qualitative description of flow show the complete distribution across the lumen instead of cross-sectional mean which preserve his profile during consecutive cycles. Concordance between values demonstrate using a twin model the sensibility (98%) of this new technology. As previously shown distension of fetal aorta adapt to flow demand and maintain constant wall shear rate.

Fetus	WSR (7)	rWDR (7)	VP (7)	Gestational age correlation
Umbilical Twin A	482±108	1.5±0.2	1	rs=0.55
Umbilical Twin B	459±121	1.9±0.7	1	rs=0.55
Aorta Twin A	453±171	1.7±0.8 *	2	*rs=0.47
Aorta Twin B	472±155	1.9±1.1*	2	*rs=0.47

*p<0.01

535

Inflammation-Induced Preterm Birth and Adverse Neurological Outcome: The Role of Exicatory Receptors.
Amy Bentz,[1] Akiva Cohen,[2] Traci Lifsted,[1] Ilana Nissim,[3] Yudkoff Mark,[3] Michal A Elovitz.[*1] *[1]OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Neurology, Children Hospital of Philadelphia, Philadelphia, PA, USA; [3]Division of Child Development, Children's Hospital of Philadelphia, Philadelphia, PA, USA.*
Objective: The presence of inflammation in a preterm birth (PTB) is associated with adverse neurological outcome in the neonate. Specifically, preterm infants from PTBs complicated by inflammation are at increased for cerebral palsy. While there has been much focus on the role of inflammatory pathways in fetal brain injury associated with PTB, we hypothesize that disruptions in neuronal development and an imbalance in excitatory/inhibitatory pathways may be a mechanism by which inflammation-induced PTB results in adverse neurological outcomes.
Methods: An established murine model of intrauterine inflammation was used. On E15, timed pregnant CD-1 dams were randomized to intrauterine infusion of saline or lipopolysaccharide (LPS) into the right uterine horn (n=4-5/treatment group). Fetal brains were harvested 6 hrs after exposure to intrauterine LPS. MRNA expression of NMDA receptor (NR) subunits was assessed by real-time PCR. Amino acid (AA) concentration was assessed using high performance liquid chromatography.
Results: In control brains, NR1 and NR2 had the lowest mRNA expression compared to NR2A, NR 2B, NR 2D, or NR 3A. Exposure to intrauterine LPS significantly up-regulated NR 2B, NR 2D, and NR 3A ($p<0.05$ for each compared to saline exposed). Intrauterine exposure to LPS resulted in at 19.6-fold in NR 1 ($p=0.016$) and a 10-fold increase in NR 2C mRNA compared to saline ($p=0.01$). AA concentrations shown in FIGURE. The branched chain AA were increased 2-fold in LPS exposed. Aspartate and glutamate were significantly decreased in fetal brains exposed to LPS ($p=0.006$ and 0.011, respectively).
Conclusions: Intrauterine inflammation results in the differential regulation of NMDA receptor sub-units as well as altering AA compositon in the fetal brains. Pertubations of neuronal function in the fetal brain may be a key to understanding adverse neurological outcomes in preterm neonates.



536

Evidence for a Novel Mechanism for Human Myometrial Activation.
David A MacIntyre,[1] Mark Read,[1] Roger Smith,[*1] Elisa Tyson,[1] Kenneth Kwek,[2] George Yeo,[2] Chan Eng-Cheng.[1] *[1]Mothers and Babies Research Centre, University of Newcastle, New South Wales, Australia; [2]KK Women's and Children's Hospital, Singapore, Singapore.*
Background: In vascular smooth muscle post-translational modifications (PTM) to small heat shock proteins (sHSPs) modulate their interaction with the actin cytoskeleton and thus contractility. The mechanisms regulating human myometrial contractility during gestation and labor are poorly understood but are also likely to involve the PTM of sHSPs.
Hypothesis: Labor is associated with PTM and altered interaction of the sHSPs and the cytoskeleton.
Aim: To identify changes in PTM and interactions of myometrial sHSPs at the time of labor.
Methods: Myometrial biopsies obtained during Caesarean Section (non-laboring; NL) or following spontaneous labor (L) were analysed quantitatively using 2D-DIGE (n=6 per group). Proteins that changed with labor were sequenced using MALDI-ToF. Immunoblotting of NL (n=14) and L (n=11) myometria was performed using antibodies to α-B-crystallin, HSP27, its phospho-isoforms (pHSP27-ser15, ser78 and ser82) and α-smooth muscle actin. Immunoprecipitation and immunofluorescence (n=5) were performed using antibodies to α-B-crystallin, HSP27 and α-smooth muscle actin.
Results: 2D-DIGE analysis demonstrated a 3.3-fold decrease in the sHSP -B-crystallin with labor. We also found specific changes in the phospho-isoforms of HSP27 between NL and L myometria. pHSP27-ser15 was 3-fold higher in L myometria whereas pHSP27-ser82 was 6.5-fold lower in L myometria. Immunoprecipitation studies revealed *in vivo* associations between HSP27 and α-B-crystallin, and HSP27 and α-smooth muscle actin in both NL and L myometria. Immunofluorescence showed high levels of co-localisation between HSP27 and α-B-crystallin in the perinuclear and cytoplasmic region of NL myometria and a relocation of HSP27 to the cytoskeleton in L myometria.
Discussion: These data support a model for human myometrial contraction in which α-B-crystallin associates with HSP27 in NL myometrium. In labor, phosphorylation of HSP27 leads to its dissociation from α-B-crystallin and subsequent relocation to the actin cytoskeleton where it stabilises filamentous actin, enabling contraction.
Conclusion: Myometrial contraction involves the regulation of fibril actin by sHSPs. These proteins may provide a rational target for the development of novel therapeutics for the treatment of preterm labor.

537

Human Myometrium Prostaglandin $F_{2\alpha}$ Receptor Gene Promoter Binds Glucocorticoid Receptor, cFos and cJun by ChIP Assay.
Begona Campos,[*] Diane Brockman, Leslie Myatt.[*] *Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cinicnnati, OH, USA.*
Prostaglandins play an important role in the initiation and maintenance of labor. PGs act by binding to their specific receptor. In human pregnancies $PGF_{2\alpha}$ is associated with stimulation of contractions in labor, acting via the prostaglandin $F_{2\alpha}$ receptor (FP). This receptor is a member of the seven transmembrane $G_{\alpha q}$ protein-coupled receptor group. Recently, Zaragoza et al. cloned and characterized the promoter region of the FP receptor. Their results showed the presence of repressor and enhancer regions in the promoter suggesting that this gene can play an important regulatory role in pregnancy.
The objective of this study was to explore the in vivo interactions between different regulatory proteins, transcriptor factors, and the $PGF_{2\alpha}$ receptor promoter using the Chromatin immunoprecipitation (ChIP) assay.

Methods: Biopsies of myometrium (n=3) were obtained during cesarean section of women in labor at term. Tissue was pulverized in liquid nitrogen, then the protein crosslinked to DNA with 1% formaldehyde. Immediately after the chromatin was isolated, DNA was sheared by enzymatic digestion. The chromatin was pre-cleared and then immunoprecipitated with antibodies against glucocorticoid receptor, cFos and cJun. The DNA was eluted and analysed by PCR. The antibodies used in this study were selected after analysis of the promoter using the Transcription Element Search System (TESS). This program predicts transcription factor binding sites in DNA sequences. It can identify binding sites using site or consensus strings and positional weight matrices from the TRANSFAC, IMD, and our CBIL-GibbsMat databases.
Results: Our results show that the glucocorticoid receptor, c Jun and cFos bind to the FP receptor promoter region, using a primer specific for the promoter region -1505 to -1340 of the 4106 bp DNA fragment of the FP promoter DNA.
Conclusions: These novel data suggest new avenues of approach for determining which factors regulate expression of the FP receptor in human myometrium and which may control the changes in the quiescent myometrial phenotype, that exists through most of gestation, to the contractile phenotype of labor.

538

Glucocorticoid Receptor, pCREB-1 and CBP Binding to the Prostaglandin Endoperoxide H Synthase (PGHS-2) Promoter in Term Amnion *In Vivo*.
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[1]Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia; [2]Gene Regulation and Expression, University of Dundee, Scotland, United Kingdom; [3]Obstetrics and Gyneacology, John Hunter Hospital, Newcastle, NSW, Australia.
PGHS-2 (PTGS2) gene activity is up-regulated in the amnion at late gestation, and the resulting increase of PGHS-2 expression is critical for generating prostaglandins that promote parturition. Using chromatin immunoprecipitation (ChIP) with fresh amnion, we have demonstrated that the proximal 1000bp region of the PGHS-2 promoter has permissive chromatin structure with highly acetylated histones H3 and H4. ChIP studies have suggested that NFκB factors bound to the 2 consensus NFκB binding sites in this region are not involved in the stimulation of PGHS-2 gene activity in term amnion *in vivo*.
Objective: Here we examined if other transcription factors, such as the glucocorticoid receptor (GR), phosphorylated cAMP-response element binding protein (pCREB-1) and CBP (CREB-binding protein, a histone acetyl-transferase), bind to the PGHS-2 promoter potentially regulating its activity.
Methods: Amnion was collected after spontaneous labor or elective Caesarean section (n=3 and 4) at term, and ChIP was performed using cross-linked chromatin from fresh tissues and antibodies for GR, pCREB-1 and CBP. The immunoprecipitated DNA was analysed by real-time PCR using 8 primer pairs distributed along the first 2500bp of the PGHS-2 promoter.
Results: We have detected GR-binding to the proximal 1000bp region of the PGHS-2 promoter ($p<0.05$, ANOVA) where chromatin structure is permissive, but no consensus GR-response sequence (GRE) is found. No GR binding was detected in the upstream restrictive chromatin region. Further, GR binding was most pronounced at 213-222 bases upstream of the transcription initiation site. Binding to this site decreased after labor ($p<0.05$. t-test). pCREB-1 has also exhibited significant binding to this site prior to labour, but not after labor. No significant binding of CBP was detected along the 2500bp region examined.
Conclusions: The data suggest that glucocorticoids may be involved directly in the control of the PGHS-2 gene promoter in the amnion before labor *in vivo*. The existence of an amnion-specific GRE or a GR complex with other factors (eg, pCREB) remain to be determined. CBP does not appear responsible for chromatin remodelling in the proximal PGHS-2 promoter region.

539

Monocyte Chemoattractant Protein-1 Integrates Mechanical and Endocrine Signals That Mediate Term and Preterm Labor.
Oksana Shynlova,[1] Anna Dorogin,[1] Stephen Lye.[*1,2,3]
[1]Samuel Lunenfeld Res Institute, Mt Sinai Hosp, Toronto, ON, Canada; [2]Obs/Gyn; [3]Physiology, University of Toronto, ON, Canada.
Pro-inflammatory cytokines contribute directly to term and preterm human labor. Previously we demonstrated that monocyte chemoattractant protein-1 (MCP-1) gene expression was dramatically upregulated in the rat myometrium prior to and during labor (when plasma progesterone (P4) levels fall). Expression of MCP-1 gene and protein was up-regulated by stretch in human endothelial and mesangial cells. Thus we **hypothesize** that (1) mechanical stretch of the uterus imposed by the growing fetus and (2) physiological withdrawal of a P4 induce myometrial MCP-1 gene expression.
Methods. We studied the effects of maintained P4 levels at term and the influence of early hormone withdrawal on MCP-1 mRNA levels. We also used unilaterally pregnant rats (*in vivo* model) and primary culture of rat myometrium smooth muscle cells (SMCs) (*in vitro* model) to study MCP-1 gene induction by static mechanical stretch. To confirm the involvement of MCP-1 in human labor we evaluated its mRNA expression in non-pregnant, preterm and term laboring myometrium. MCP-1 gene expression analysis was performed by real-time RT-PCR.
Results. MCP-1 was expressed at very low levels in gravid horn from early gestational myometrium with a dramatic increase observed before and during labor. In contrast to the gravid horn, expression of the chemokine in the non-gravid horn was very low throughout pregnancy, implying a role for uterine stretch. To examine the effects of elevated P4 during late gestation, rats were injected with P4 or vehicle starting from d20 of pregnancy. Administration of P4 caused a failure in the normal elevation of the MCP-1 mRNA levels on d22-d23 and delayed the onset of labor. In contrast, treatment of pregnant rats with P4 receptor antagonist RU486 on d19 induced preterm labor on d20 and a premature increase in mRNA levels of MCP-1. Static mechanical stretch of freshly isolated primary rat myometrial SMCs (25% for 30 min) induces (1) a rapid increase in MCP-1 gene expression, an effect that was (2) enhanced by pro-inflammatory cytokine IL-1b but (3) repressed by pretreatment with P4. MCP-1 mRNA levels were increased in human myometrium during term and preterm labor compared to non-pregnant control.
Conclusion: These data indicate that MCP-1 serves to integrate mechanical and endocrine signals that induce labor and may represent a novel target for human preterm tocolytic therapy.

540

A Novel Mode of Action for Oxytocin and Cyclic Nucleotides in the Regulation of Myometrial Contractility.
Elisa Tyson, David A MacIntyre, Roger Smith,[*] Eng-Cheng Chan, Mark Read. *Mothers and Babies Research Centre, Newcastle, NSW, Australia.*
Background The small heat shock proteins (sHSP) 27 and 20 are implicated in modulating smooth muscle contraction and relaxation. p38MAPK-induced phosphorylation of HSP27 enhances contraction in smooth muscle. Conversely, cyclic nucleotide induced phosphorylation of HSP20 relaxes smooth muscle, in part by opposing the effects of HSP27. The role of the sHSPs in human myometrium, particularly with the transition of myometrial smooth muscle to a contractile phenotype at labour, has not been examined. Recently, we demonstrated an increase in total HSP27 protein throughout human gestation and an increase in phospho-HSP27-serine15 with labour.
Hypothesis That oxytocin, a powerful uterotonic, will induce phosphorylation of HSP27 in myometrium *in vitro,* whereas increases in cAMP and cGMP, as occur with some tocolytics, will cause an increase in phospho-HSP20.
Aim To examine in human myometrial strips, the effects of oxytocin, forskolin, sodium nitroprusside, rolipram (cAMP phosphodiesterase (PDE) inhibitor) and vardenafil (cGMP PDE5 inhibitor) on: (i) myometrial contractility, (ii) HSP27 phosphorylation and (iii) HSP20 phosphorylation.
Methods Myometrium was obtained at elective Caesarean section. Force generation in spontaneously contracting strips was measured following exposure to either a single sub-maximal or cumulative log doses of each drug. Following treatment, protein from strips was extracted and resolved by 1D and/or 2D SDS-PAGE. Western immuno-blotting was performed using antibodies for total and phosphorylated forms of HSP20 and HSP27.
Results Oxytocin caused a significant increase in pHSP27-ser15 (n=5, $P<0.05$), functionally associated with a 133±25% increase in myometrial tension. Phospho-HSP27-ser78 and 82 were unchanged. Oxytocin did not alter pHSP20. Forskolin, SNP, rolipram and vardenafil all significantly increased pHSP20 (n=4, $P<0.05$), associated with an 84-100% reduction in tension, however there was no change in total or phospho-HSP27.
Conclusion Oxytocin-stimulated contraction of human myometrium induces a specific phosphorylation of HSP27 on serine 15. Agents that elevate cyclic nucleotides increase phosphorylation of HSP20, associated with myometrial relaxation. These data provide functional and biochemical support for a novel role for phosphorylated sHSPs in modulating human myometrial contractility.

541

PSF and the Regulation of the Progesterone Receptor Gene in the Human Myometrium during Pregnancy. Alison J Tyson-Capper, Stephen C Robson.* *Surgical & Reproductive Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom.*

There is increasing evidence to suggest that specific isoforms of progesterone receptors (PRs) are differentially expressed within the myometrium at term and at the onset of labour. The promoter regions for the PRs contain various functional transcription factor sites (PR-B has one Sp1 site and PR-A has two Sp1 sites and a half ERE site), however, the regulatory processes that control expression of PRs within the myometrium are not fully understood. PSF, a multi-functional RNA and DNA binding protein that increases in the upper uterine region at term, has an inhibitory effect on the expression of PRs in human myometrial cell cultures.

Objectives: To investigate how PSF contributes to the regulation of PR expression by identifying nuclear co-activators/co-repressors that interact with PSF and to determine potential transcription factor binding sites for PSF and its co-factors within the regulatory regions of the PR promoter sequences.

Methods: Co-immunoprecipitations (Co-IPs), immunofluorescence, electrophoretic mobility shift assays (EMSAs) and DNA affinity immunoprecipitation assays (DAPAs).

Results: Co-IPs indicated that PSF directly interacts with Sp proteins, histone deacetylases, mSin3A, and MEF-2. Dual-labelling immunofluoresence confirmed co-localisation of these nuclear co-factors within myometrial nuclei. We identified a potential MEF-2 transcription factor site ($CTA(A/T_4)$ upstream of the Sp1 sites within the PR promoter; to determine whether the MEF-2 site is functional oligonucleotides were designed containing the MEF-2 sequence (and a mutated MEF-2) and EMSAs performed. Nuclear myometrial proteins do interact with the MEF-2 sequence and the addition of specific antibodies to MEF-2 or PSF resulted in a supershift of the MEF-2 oligonucleotides. DAPAs indicated that PSF, MEF-2, HDAC1, HDAC2, mSin3A and another co-factor RbAp48, are part of the multi-protein complex bound to the MEF-2 sequence. DAPAs show that PSF, Sp1, HDAC1, HDAC2 and RbAp48 also interact with the Sp1 site within the PR-B promoter. Finally, Co-IPs showed that PSF also interacts with specific PRs and progesterone response elements within myometrial nuclei.

Conclusions: Our data suggests that PSF and co-factors may play a role in PR expression and progesterone mediated gene regulation within the human myometrium. Whether PSF, via the MEF-2 binding site and/or Sp1 binding sites influences PR expression in the myometrium will now be investigated.

542

Expression of Membrane Progesterone Receptors in the Human Pregnancy Myometrium: Changes with Gestation and the Onset of Labor. Amy A Merlino,[1] Huiqing Tan,[2] Li Juan Yi,[2] Brian Mercer,[*1] Sam Mesiano.[*2] *[1]Department of Reproductive Biology, CASE – MetroHealth Medical Center, Cleveland, OH, USA; [2]Department of Reproductive Biology, CASE – University Hospitals, Cleveland, OH, USA.*

OBJECTIVE: Progesterone maintains pregnancy by promoting myometrial quiescence through genomic and non-genomic mechanisms, and labor is thought to be initiated by progesterone withdrawal. The role of non-genomic progesterone actions in human pregnancy and parturition are not clearly understood. Non-genomic progesterone actions are mediated by membrane-bound PRs (mPRs): mPRα, mPRβ, mPRγ, and progesterone receptor membrane components -1 and -2 (PGRMC-1 and PGRMC-2). The goal of this study was to measure the extent of mPR expression in biopsy specimens of human myometrium obtained at cesarean delivery, and to determine whether expression changes with advancing gestation or the onset of labor.

STUDY DESIGN: Lower uterine segment myometrial biopsies were obtained at the time of delivery from 35 consenting women who were preterm not in labor (PTNIL: n = 7), term not in labor (TNIL: n=12) and term in labor (TIL: n=16). Relative abundance of mRNAs encoding mPRα, mPRβ, mPRγ and PGRMC-1, PGRMC-2 were determined by quantitative RT-PCR, normalized to mRNAs encoding the constitutively expressed myometrial-specific gene h-caldesmon, and compared between the 3 groups.

RESULTS: In all samples mRNAs encoding PGRMC-1 and PGRMC-2 were more abundant than mPRα mRNA, which was greater than mPRβ and mPRγ (least abundant) mRNAs. There were no differences in mRNA levels for each mPR between PTNIL and TNIL biopsies. However, labor at term (TIL group) was associated with a 2 to 3-fold increase in relative abundance of mRNAs encoding PGRMC-1 (p=0.04) and PGRMC-2 (p=0.029) compared with TNIL. Expression of the other receptors was not affected by labor status.

CONCLUSION: These data demonstrate for the first time that the human pregnancy myometrium (at least from the lower uterine segment) expresses robust levels of PGRMC-1, PGRMC-2 and mPRa, suggesting that progesterone may influence its contractility non-genomically via these receptors. The functional significance of the labor-associated increases in PGRMC-1 and PGRMC-2 is unclear. Changes in expression of these receptors during pregnancy may be important for the hormonal control of parturition.

543

MicroRNA Expression Profiling of the Human Uterine Cervix in Term Labor. Sonia Hassan,[1,2] Roberto Romero,[*1,3] Beth Pineles,[1] Adi L Tarca,[1,4] Daniel Montenegro,[1] Jimmy Espinoza,[1,2] Pooja Mittal,[2] Shali Mazaki-Tovi,[2] Juan Pedro Kusanovic,[1] Lara Friel,[2] Francesca Gotsch,[1] Sorin Draghici,[4] Chong Jai Kim.[1,5] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [3]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [4]Department of Computer Science, Wayne State University, Detroit, MI, USA; [5]Department of Pathology, Wayne State University, Detroit, MI, USA.*

OBJECTIVE: MicroRNAs (miRNA) are noncoding RNAs involved in post-transcriptional regulation of target genes. miRNAs play a role in embryonic development, granulocyte differentiation and oncogenesis. The objective of this study was to determine whether cervical ripening/dilatation during human parturition is associated with changes in the miRNA expression profile.

METHODS: The miRNA expression pattern of cervical tissue was characterized using miRCURY™ LUNA microarrays. Samples were collected at term from patients without labor (TNL; n=9) and after spontaneous labor (TL; n=8). P-values for differential expression derived from a moderated t-test model were adjusted for multiple comparisons. Results were confirmed using qRT-PCR.

RESULTS: 1) Microarray analysis revealed that 226 miRNAs were expressed in human cervical tissue at term; 2) Unique miRNAs (miR-223, miR-34b, miR-34c) were differentially expressed in the cervical tissue of women who underwent spontaneous labor compared to those not in labor (false discovery rate < 0.05); 3) miR-223 (fold change FC= 4.8), miR-34b (FC=3.3), and miR-34c (FC=8.2) were up-regulated in the cervical tissue of women in labor when compared to that of patients not in labor; 4) Targeted qRT-PCR assays confirmed these findings [miR-223 (FC=5.7, p<0.001), miR-34b (FC=4.5, p<0.001), miR-34c (FC=6.2, p<0.001)]. We also obtained confirmation of non-differentially regulated miRNAs with qRT-PCR.

CONCLUSION: 1) We report for the first time that miRNAs are differentially regulated in the human uterine cervix during spontaneous labor; 2) Cervical remodeling after term labor was associated with changes in the miRNA expression pattern which is characterized by over-expression of miR-223, miR-34b, and miR-34c; 3) We propose that miRNAs participate in post-transcriptional regulation of genes involved in cervical remodeling.

544

The Role of LOX-1 Receptor in the Pathophysiology of Preeclampsia. Sowndramalingam Sankaralingam,[1] Yi Xu,[1] Tatsuya Sawamura,[2] Sandra T Davidge.[*3] *[1]Physiology, University of Alberta, Edmonton, AB, Canada; [2]Vascular Physiology, National Cardiovascular Center Research Institute, Osaka, Japan; [3]Obstetrics/Gynecology & Physiology, University of Alberta, Edmonton, AB, Canada.*

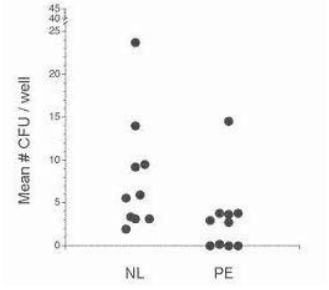
Background: Preeclampsia (PE) is characterized by the onset of hypertension and proteinuria after the 20th week of gestation. Oxidative stress may mediate vascular dysfunction in PE. We have shown evidence of peroxynitrite ($ONOO^-$) formation in the vasculature of PE women. Furthermore, endothelial cells incubated with plasma from PE women generate $ONOO^-$ as measured by nitrotyrosine staining. There is also increased levels of oxidized low density lipoprotein (oxLDL) in the plasma of PE women. oxLDL binds to the lectin like oxLDL receptor -1 (LOX-1) on the endothelium of blood vessels and generates superoxide (O_2^-) *via* NADPH oxidase. Our preliminary data shows increased LOX-1 receptor expression and presence of oxLDL in the omental arteries of women with PE, while there was negligible expression in the normotensive pregnant (PREG) women. However, the mechanisms of increased LOX-1 receptor in PE is unknown. We hypothesize that circulating factors in PE plasma will upregulate the LOX-1 receptor resulting in enhanced O_2^- generation and subsequently $ONOO^-$. Furthermore, $ONOO^-$ will provide a feed forward loop to upregulate LOX-1. **Methods:** Human umbilical vein endothelial cells (HUVECs) were treated with 2% plasma (*n*=3-5 per group) from PREG or PE women for 24 hours. LOX-1 receptor expression was assessed by Western blot. O_2^- generation in response to plasma was measured

using dihyrdoethidium, in the presence of a monoclonal blocking antibody to LOX-1 receptor (mAbLOX-1) or apocynin, an NADPH oxidase inhibitor. In some experiments, LOX-1 was assessed in HUVECs treated with $ONOO^-$ (25 μM) for 6 h. **Results:** PE plasma tended to increase (~ 30±1.5 %, $p<0.07$) LOX-1 receptor expression in HUVECs compared to treatment with PREG plasma. PE plasma significantly increased O_2^- generation when compared to treatment with PREG plasma (130±3 *vs*. 26±2 Arbitrary Units (A.U.), $p<0.001$), that was inhibited by mAbLOX-1 or apocynin (65±3 and 63±2 A.U., respectively $p<0.001$). Furthermore, $ONOO^-$ increased LOX-1 protein expression ($p<0.01$). **Conclusions and Speculations:** In preeclampsia, LOX-1 receptor and NADPH oxidase may play a role in O_2^- and subsequently $ONOO^-$ generation in response to plasma, ultimately resulting in vascular dysfunction. LOX-1 receptor antagonism may be a potential therapeutic target in PE.

545

Clonogenic Endothelial Progenitor Cells Are Decreased in Women with Preeclampsia. Carol Lin,[1] Augustine Rajakumar,[*1,2] Vivek Verma,[2] Nina Markovic,[2,3] Roberta B Ness,[2,3] Daniel Plymire,[2] Carl A Hubel.[*1,2] *[1]Dept. of OB/GYN and Reproductive Sciences; [2]Magee-Womens Research Institute, Univ. of Pittsburgh School of Medicine; [3]Dept. of Epidemiology, Univ. of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

Background: Bone marrow-derived endothelial progenitor cells (EPCs) enter the systemic circulation to replace defective or injured mature endothelial cells. Suppression of EPCs may have detrimental cardiovascular effects. **Objective:** We asked if numbers of early outgrowth EPC colony-forming units (CFUs) derived from maternal blood are decreased in women with preeclampsia compared to normal pregnancy. **Methods:** Primigravid non-smokers with normal pregnancy (NL, n=10) or preeclampsia (PE, n=10) and singleton gestation were studied during the 3rd trimester. Patients with chronic hypertension, other metabolic disorders, or acute inflammatory conditions were excluded. Groups did not differ by race, prepregnancy body mass index or gestational age at time of venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated from maternal blood by Ficoll density centrifugation. To eliminate mature circulating endothelial cells, PBMCs were pre-plated on fibronectin-coated 6-well plates ($5x10^6$ cells/well) in Endocult Medium (StemCell Technologies). After 48 hours, nonadherent cells were recollected and 1 million cells replated into fibronectin-coated 24-well plates. CFUs were manually counted 72 hours later in a minimum of 4 wells/patient. Groups were compared by Mann-Whitney U test. The endothelial lineage of CFUs was confirmed by co-immunostaining with DiI-labeled acetylated LDL and FITC-labeled Ulex europaeus lectin. **Results:** Median EPC-CFU counts were half normal in preeclampsia [PE 2.9 (interquartile range 0.0 to 3.8), NL 5.8 (3.2 to 9.5), $p=0.041$]. Scatterplot shows average number of CFUs per well for each patient.



Conclusions: Numbers of circulating, clonogenic EPCs are decreased in women with preeclampsia; this deficit may be a factor in the pathogenesis of the disease. Funded in part by the PA Dept. of Health and by NIH R21HD49453, MO1RR00056 and PO1HD30367.

546

Prepregnant Low Plasma Volume Interferes with the Cardiac Adaptation to Pregnancy. Silvia Andrietti,[1,3] Arnold Kruse,[1] Simone Sep,[1] Maurizio Marco Anceschi,[3] Marc Spaanderman,[2] Louis Peeters.[*1] *[1]Ob-Gyn, Univ Hosp Maastricht, Maastricht, Netherlands; [2]Ob-Gyn, UMC St. Radboud, Nijmegen, Netherlands; [3]Ob-Gyn, Univ La Sapienza, Rome, Italy.*

Background: Formerly preeclamptic women with a subnormal plasma volume (LPV) have a 3 times higher chance to develop recurrent hypertensive disorder in their next pregnancy as compared to their counterparts with a normal plasma volume (NPV) (BJOG, 2003;110:1001). In this study we tested the hypothesis that LPV have a reduced cardiac capacity to adapt to pregnancy.

Methods: In 12 LPV (PV < 45 mL. [kg lean body mass]$^{-1}$), 26 NPV (PV > 52 mL) and in 9 parous controls (CONTR), we measured the following echocardiographic parameters along with blood pressures at least 5 months post partum, at days 5 (baseline) and 18 of the menstrual cycle and again at pregnancy weeks 5 and 7 in their next pregnancy: Left atrial diameter (LAD), left ventricle enddiastolic - (EDV) and endsystolic volumes, left ventricle mass (LVM), ejection fraction (EF), heart rate (HR), stroke volume, cardiac output, E/A ratio (defined as the ratio between the peak mitral flow in early diastole [E] and during atrial contraction [A]), diastolic - (DBP), systolic - (SBP), mean arterial pressure (MAP), pulse pressure (PP) and global compliance (GC, defined as the ratio of SV and PP). All participants were normotensive (no medication) and seemingly healthy. Groups were compared with each other by means of the Mann-Whitney-U Test, Wilcoxon Signed Rank Test or Friedman One-Way ANOVA, whenever appropriate. Data are presented as median (IQR).

Results: Baseline parameters in LPV differed from those in NPV only by a lower GC (see table). However, the response to pregnancy in the LPV group differed from that in both NPV and CONTR by a rise in LAD to 39 (4) mm ($p = 0.017$) and HR to 74 (14) bpm, ($p = 0.033$), and a delayed decrease in DBP.

Conclusion: The difference in cardiac adaptation to pregnancy between LPV and NPV suggest impaired diastolic cardiac function and a higher cardiovascular sympathetic tone in LPV relative to NPV and therefore, supports our hypothesis.

	Age (y)	BMI ($kg.m^2$)	PV (mL/lbm)	LAD (mm)	EDV (mL)	LVM (g)	E/A ratio	Heart rate	GC
CONTR	32 (4)	21.2 (3.4)	54 (10)	34 (6)	88 (29)	124 (30)	1.65 (0.74)	72 (13)	1.7 (0.4)
NPV	32 (5)	22.5 (4.8)	54 (4)	36 (3)	97 (14)	134 (23)	1.70 (0.40)	67 (16)	1.7 (0.2)
LPV	28 (5)	26.2 (4.4)	43 (3)*	37 (5)	97 (51)	139 (43)	1.82 (0.7)	64 (14)	1.4 (0.4)*

Demographic - and baseline echocardiographic variables. Data are presented as median (IQR).
* LPV different from NPV ($p< 0.05$).

547

24 Hour Urinary Protein Levels Prior to and during a First Pregnancy. Ira Bernstein,[*1] Adrienne Schonberg,[1] Robert Shapiro,[2] Beth Bouchard,[3] Alan Segal.[4] *[1]OB/GYN, Univ of VT, Burlington, VT, USA; [2]Neurology, Univ of VT, Burlington, VT, USA; [3]Biochemistry, Univ of VT, Burlington, VT, USA; [4]Medicine, Univ of VT, Burlington, VT, USA.*

BACKGROUND: The diagnostic criteria for preeclampsia require 24 hour urinary protein levels of at least 300 mg in the second half of pregnancy. As part of a study of prepregnancy physiology that might contribute to the development of preeclampsia we characterized 24 hour urine profiles in nulligravid women prior to and then during the course of their first pregnancy.

METHODS: We examined 50 healthy young normotensive women during the follicular phase of the menstrual cycle (MC). Thirteen of these women were studied again at 11-16 weeks of subsequent pregnancies (EP) and 6 of these women have been studied at 30-34 weeks (LP) for a total of 69 collections. None of these women received a clinical diagnosis of hypertension or preeclampsia. Collections were performed during inpatient stays in our General Clinical Research Center after 3 days of dietary control. During the hospital stay we measured oral intake, controlling calories, and 24 hour urinary volume, protein, creatinine clearance and sodium. Twenty four hour protein was measured using the VITROS UPRO Slides kit (Ortho Diagnostics, Raritan, NJ). Data is expressed as mean $\pm$ standard deviation.

RESULTS: Subjects were 29.4 $\pm$ 4.7 years old with a BMI of 22.8 $\pm$ 3.7 kg/m^2 at prepregnant studies. Urinary volumes were MC: 2,439 $\pm$ 1,014, EP: 2,591 $\pm$ 890 and LP 4,309 $\pm$ 1,688 mL. The mean 24 hour urinary protein during MC studies was 251 $\pm$ 163 mg. The majority of results were above the normal limit (150 mg) for nonpregnant 24 hour urinary protein (70%, 35/50). The mean 24 hour urine protein at EP was 296 $\pm$ 109 mg (range 84-477 mg): LP was 592 $\pm$ 242 mg (range 301-882). Seventy four percent (14/19) of pregnancy results exceeded 300 mg/24 hours. The mean creatinine clearance prior to pregnancy was 114 $\pm$ 20 mL/min (range 73-171). Mean prepregnancy levels of urinary sodium were 47 $\pm$ 52 mEq/24 hours.

CONCLUSIONS: We have demonstrated "abnormal" 24 hour urinary protein concentrations in a majority of healthy normotensive nulligravidas and subjects studied during first pregnancies. These results, obtained from commercial analysis under highly supervised conditions, suggest that the current threshold for 24 hour urine protein employed in the diagnosis of preeclampsia may need reevaluation. Supported by NIH RO-1 HL71944.

FRIDAY

548

Hypoxia and TGFβs Regulate Endoglin Expression in Human Placenta. Yoav Yinon,[1] Ori Nevo,[1] Jing Xu,[1] Alessandro Rolfo,[1] Isabella Caniggia.*[1,2] *[1]Depart. of Ob/Gyn, Mount Sinai Hospital; [2]Depart. of Physiology, University of Toronto, Toronto, ON, Canada.*

Objective: Endoglin, a co-receptor for transforming growth factor (TGF)-β1 and β3, is expressed in the human placenta where it plays a role in regulating early events of trophoblast differentiation. Recent evidence has indicated that in preeclamptic placentae endoglin expression is elevated and this is associated with high circulatory levels of its soluble form. Since preeclampsia may be the result of impaired oxygenation, we examined the effect of oxygen on endoglin expression using physiological and pathological models of placental hypoxia. Since TGFβ3 expression in the placenta is regulated by oxygen, we also examined the effect of TGFβ1 and TGFβ3 on endoglin expression.

Methods: Human placental tissue from first trimester and from discordant dichorionic and monochorionic twins (n=10) and age matched normal twins (n=5) were used as physiological and pathological models of placental hypoxia. In all twins the discordancy was above 25% and absence of end diastolic velocity was documented in the growth-restricted twin. Endoglin mRNA expression was measured by quantitative PCR analysis. Protein expression was measured by Western Blot analysis using endoglin antibodies. Villous explants (5-8 wks) were used to test the effect of both oxygen and TGFβ1 and TGFβ3 on endoglin expression.

Results: Immunoblot analysis indicated that endoglin expression was high at 5-7 wks of gestation, when oxygen tension is low and decreased after 10 wks when oxygen tension increases. Real-time PCR showed significantly increased endoglin transcripts in IUGR discordant twins compared to their normal co-twins and to control non-discordant twins. Consistent with the mRNA findings, we observed that protein levels of both membrane and soluble endoglin were higher in the IUGR twin placentae relative to both the control co-twin and the normal twins.Exposure of villous explants to low oxygen (3% O_2) resulted in elevated expression of endoglin compared to standard conditions (20% O_2). Moreover, addition of TGF β1 and TGFβ3 to villous explants also increased the expression of both membrane and soluble endoglin compared to non-treated explants.

Conclusions: Our results demonstrate that oxygen regulates the expression of endoglin possibly via a mechanism that involves TGFβs. Reduced placental perfusion leading to placental hypoxia may contribute to the increased expression of endoglin in IUGR discordant twins. (Supported by CIHR)

549

Increased Placental Expression of Heat Shock Protein Precursor Gp96 in Preeclampsia. Rose P Webster, Brad A Pitzer, Victoria H Roberts, Diane E Brockman, Leslie Myatt.* *OB/GYN, University of Cincinnati, Cincinnati, OH, USA.*

Introduction: Gp96, the heat shock protein 90 precursor or endoplasmic reticulum counterpart of Hsp90 has been shown to be induced by a variety of stress conditions and to function as a molecular chaperone. Its role in innate and adaptive immunity is of particular relevance to preeclampsia, since abnormal activation of of the immune system has also been suggested to be responsible for the etiology of pre-eclampsia. We have here used both proteomic technologies and the traditional methods of western blot and immunohistochemistry to examine the relevance of placental Gp96 in preeclampsia.

Objective: Our objective was to examine localization of Gp96 in the placenta and its alteration in preeclampsia using 2DE and western blot.

Methods: Villous tissue from normal and preeclamptic patients was lysed at 4ºC in lysis buffer (2% CHAPS, 20 mM Tris pH 7.5, 1mM EDTA, 1 mM EGTA, 200 uM AEBSF and protease inhibitor). The lysate was centrifuged at 20,000xg at 4ºC for 5 min and the supernatant was used for all experiments. 2DE analysis (n=3) was carried out to detect proteins altered in preeclampsia and then identified by LC-MS. Western blot (n=6) and immunohistochemistry (n=6) was carried out using anti-Gp96 antibody.

Results: 2DE analysis showed that intensity of protein spot number 48 was over 6 fold higher in preeclampsia. LC-MS identified 5 peptides that matched Gp96 in a database search using Mascot software. Immunoblot analyses for Gp96 also demonstrated significantly greater amounts of the protein in the preeclamptic group as compared to normotensive ($p<0.05$) confirming the 2D finding. Immunostaining of the placental tissue localized Gp96 predominantly to the extravillous trophoblasts (EVT) and ST layer. Overall, intense staining was observed in the EVT in all the placentae. There was a distinct trend towards increased staining in the ST layer and endothelium of the preeclamptic group as against the normal**.** Stromal staining was weak in all samples.

Conclusions: Data presented here shows that Gp96 expression is significantly ($p<0.05$) increased in preeclampsia. The roles of Gp96 in protein homeostasis and cell differentiation have not been well studied. As a general rule Gp96 is induced during ischemic injury and Hsp90 is induced as a result of the oxidative stress resulting from reperfusion following ischemia both in the brain and kidney. It remains to be seen whether such an association exists in preeclampsia.

550

Identification of Antigenically Distinct, Hypoxia-Responsive sFLT Isoforms in Conditioned Media of Placental Explants in Culture and in Plasma from Normotensive and Preeclamptic Women. Augustine Rajakumar,* Eiji Shibata, Frauke von Versen-Hoynck, Daniel Plymire, Ashi Daftary, Robert Powers,* Carl Hubel.* *Magee-Womens Research Institute, Dept. of Ob/Gyn and Repro. Sciences, Univ of Pittsburgh School of Medicine, Pittsburgh, PA, USA.*

Objective: Soluble VEGF receptor, sFlt has been implicated in the pathogenesis of preeclampsia. Previous data suggest that maternal mononuclear cells are an important extra-placental source of circulating sFlt in women with preeclampsia (PE). The present study quantified the ability of placental explants from normotensive pregnant (NP) and PE women to produce sFlt under hypoxic and nonhypoxic tissue culture conditions. We also tested the hypothesis that multiple isoforms of sFlt would be present both in the conditioned medium and plasma.

Methods: The diagnosis of PE was based on the criteria of the Working Group Report on High Blood Pressure in Pregnancy (2003). Placental villous explants (NP, n=4 and PE, n=5) were exposed to 21% oxygen or 2% oxygen under tissue culture conditions for 24 hrs. Soluble Flt in the conditioned media was purified using heparin-agarose and was subjected to Western blot analysis using four different Flt and sFlt antibodies (sc-316, Santa Cruz; V4262, Sigma; 36-1100, Zymed; AF321, R&D Systems). Similar studies were carried out using plasma samples (n=4 each of NP and PE). Additionally immunoprecipitation followed by ELISA (R&D Systems) and Western analysis were carried out using plasma samples (n=6) selected based on their elevated sFlt concentration. We employed Flt-1, VEGFR-2 and sFlt antibodies to investigate the protein-protein interaction between these receptors.

Results: Under normal culture conditions, concentrations of sFlt in conditioned media from PE explants [mean 9000 pg/ml (2420 to 33300 range)] were marginally higher ($P < 0.07$) compared to NP explants [3880 pg/ml (2500 to 5475 range)]. Hypoxia upregulated sFlt protein (1.5 fold) in both groups. In addition to the 100 Kd sFlt protein described in the literature, we identified 185, 150, 140, 85, 75, and 60 Kd Flt immuno-reactive proteins. Except the 140 Kd sFlt, other isoforms appear antigenically different from the 100 Kd sFlt protein. Immunoprecipitation studies indicated that 10% of circulating sFlt is bound to either Flt-1 (soluble Flt 185) or VEGFR-2.

Conclusion: Placental production of sFlt is highly variable and exhibit multiple isoforms.

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551

Abnormalities in Angiogenic Peptides at Three Time Points in Diabetic Pregnancies. Calvin J Hobel,* Dotun Ogunyemi, Chander P Arora, Meenu Sandhu. *Obstetrics & Gynecology, Cedars-Sinai Medical Center, Burns and Allen Research Institute, Los Angeles, CA, USA.*

OBJECTIVE: To study the biochemical markers of angiogenesis in patients with diabetes during pregnancy.

HYPOTHESIS: Oxidative stress in diabetic pregnancies alters endothelial regulation.

INTRODUCTION: Diabetes may cause an imbalance of angiogenic factors during pregnancy. High plasma levels of soluble fms-like tyrosine kinase 1 (sFlt-1), the VEGF receptor-1, which increases during hypoxic conditions and binds to vascular endothelial growth factor (VEGF) leading to reduced levels of this peptide. This may affect maternal - placental development in diabetes patients.

METHODS: We identified 26 women with diabetes during pregnancy from a cohort of 524 women participating in a Behavior in Pregnancy Study (BIPS). Urinary levels of VEGF and plasma levels of sFlt-1 were measured by ELISA (R&D Systems, Minneapolis, MN). Samples from these patients and 26 matched controls were assayed during three visits, T1 (18-20 wks), T2 (24-28wks) and T3 (34-36 wks).

RESULTS: Among controls urinary VEGF levels increased progressively at T1, T2 and T3 during pregnancy (219.6 ± 17.2 pg/ml,256.2 ± 18.8 pg/ml and 330.2 ± 16.7 pg/ml respectively). In women with diabetes, the levels were

highest at T1 but declined at T2 and T3 (T1: 181.4 ± 12.3 pg/ml, T2: 131.9 ± 9.6 pg/ml, T3:127.0 ± 18.1 pg/ml). However, at all three visits these levels were significantly lower among women with diabetes in comparison to the control group (p= .001). In contrast, plasma sFlt-1 levels progressively increased over time in controls (T1: 478.9 ± 17.8 pg/ml, T2: 570.8 ± 24.5 pg/ml, T3: 791.9 ± 22.9) as well as in women with diabetes (T1: 1018.8 ± 48.8 pg/ml, T2: 1713.6 ± 41.7 pg/ml, T3: 2039.7± 34.0 pg/ml). The levels in later group were significantly higher during all three time periods (p<.001).
CONCLUSION: This is the first study to show prospectively that the angiogenic peptide VEGF is significantly reduced in diabetics as early as mid pregnancy. These reduced levels are the result of a significant elevation in its soluble receptor (sFlt-1) leading to an increasing decrement in the ratio of VEGF and sFlt-1 as the pregnancy progresses. This suggests that the diabetic state of abnormal glucose metabolism and its known role as an oxidative stressor can significantly alter these two peptides. VEGF and sFlt-1 may be of value as markers of the degree of oxidative stress and glucose homeostasis in diabetic pregnancies.

552

Demonstration of Pluripotent Stem Cells in the Adult Human Endometrium by In Vitro Chondrogenesis. Erin F Wolff, Hongling Du, Andrew B Wolff, Hugh S Taylor.* *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objectives: Stem cells have remarkable regenerative potential; many adult tissues harbor cells with extensive plasticity. We hypothesized that, in addition to endogenous stem cells restricted to an endometrial cell fate, human endometrium contains stem cells with the ability to differentiate to non-endometrial cell types. Here we demonstrate pluripotent stem cells in human endometrium by inducing *in vitro* chondrogenic differentiation.
Methods: Proliferative endometrium was initially prepared as standard for endometrial stromal cell culture including filtering through a 73 um sieve, cultured in DMEM with 10% FBS and 1% Antibiotic/Antimycotic to confluence. After the second passage, cells were centrifuged and transferred to 15 ml conical tubes. Six cell pellets were cultured in phenol red free DMEM, 10% charcoal stripped calf serum, and 1% Antibiotic/Antimycotic and another six in chondrogenic induction media containing L-Ascorbic Acid, Dexamethasone, and TGF ß2. Cultures were maintained for 3-21 days. Myometrial and tubal cells served as negative controls. Cell pellets were then analyzed for articular cartilage markers by staining with Alcian Blue and Toluidine Blue to identify sulfated glycosaminoglycans. Immunohistochemistry was performed for Type II Collagen. Samples were compared to control cell pellets cultured without chondrogenic agents.
Results: After 7 days, endometrial stromal cell pellet cultures treated with chondrogenic media demonstrated approximately 1% cells with distinctive chondrocyte features. These cells stained brightly with Toluidine Blue and extracellularly with Alcian Blue, both specific for the sulfated glycosaminoglycans secreted by articular chondrocytes. Collagen II expression, characteristic of articular chondrocytes, was demonstrated by immunohistochemistry in the cytoplasmic region of these cells. Cells cultured without induction agents were unable to form a pellet, dispersed in media and did not express sulfated glycosaminoglycans or Collagen II. Nor were any of the chondrocyte markers expressed after culturing myometrium or tubal epithelium with or without the induction agents.
Conclusions: This is the first demonstration of *in vitro* pluripotent potential of putative adult human endometrial stem cells by inducing differentiation along a chondrogenic pathway. Endometrium may serve as a reservoir for stem cells with considerable plasticity.

553

Bone Marrow Stem Cell Transplantation Rescues Long-Term Fertility in Chemotherapy-Treated Females. Ho-Joon Lee, Kaisa Selesniemi, Yuich Niikura, T Niikura, R Klein, D Dombkowski, Jonathan L Tilly.* *Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA, USA.*
Introduction: Donor-derived immature oocytes are generated in chemotherapy (CTx)-conditioned female mice after bone marrow (BM) transplantation (BMT) (*Cell* 2005 122:303). However, a new study has claimed that ovulated eggs are not derived from BM, and that the BM-derived immature oocytes identified previously are transplanted immune ($CD45^+$) cells (*Nature* 2006 441:1109). **Objective:** To assess the effects of BMT on long-term fertility following CTx, and to test if donor BM-derived oocytes are misidentified $CD45^+$ cells. **Methods:** Adult female mice (129/Sv, FVB) were given CTx, followed by BMT 1 wk later using coat color-mismatched (C57BL/6) females as donors. After housing with C57BL/6 males, the number of successful pregnancies was recorded. For donor cell tracking, BM from germline-specific GFP-transgenic mice was injected into CTx-treated wild-type recipients. Immune ($CD45^+$) cells were sorted from blood and analyzed for germline marker expression. **Results:** All (10/10) non-treated females achieved 5, and 80% achieved 6, pregnancies over the 7-mo trial. Mice given CTx without BMT became infertile, with the majority (10/13) achieving 3 or less, and none achieving 6, pregnancies. In mice receiving BMT 1 wk after CTx, one was sterilized. However, 90% (9/10) achieved at least 4, 80% achieved 5, and 70% achieved 6, pregnancies over the 7-mo trial. All offspring (n=325) produced by the transplanted females were from the recipient germline, although donor cell tracking showed that donor-derived (GFP^+) immature oocytes were present in recipient females. Finally, in contrast to the previously reported co-expression of germline markers by these GFP^+ oocytes, $CD45^+$ cells did not express germline markers. **Conclusions:** BMT rescues fertility in CTx-treated adult female mice. Although donor BM-derived immature oocytes (which are not misidentified $CD45^+$ cells) are generated after BMT, all offspring produced by these females are recipient-derived. These data support the existence of stem cells in BM with germline potential, but indicate that oocytes formed by BM-derived cells rarely, if at all, become competent for ovulation and fertilization. Nonetheless, BMT rescues long-term fertility in CTx-treated females, through mechanisms that involve revival of a small cohort of existing oocytes or a reactivation of host oogenesis. **Support:** NIH R37-AG012279, R01-AG024999.

554

Human Amnion – A Novel Source of Pluripotent Stem Cells for Regenerative and Repartive Medicine. Ursula Manuelpillai, Sivakami Ilancheran, Euan M Wallace. (SPON: Beverley J Vollenhoven). *Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia.*
Objective: The amnion is the inner of two membranes surrounding the fetus. Amniotic epithelial cells (AEC) are formed from pluripotent embryonic epiblast cells prior to gastrulation. Even at term the amnion could harbour pluripotent stem cells and/or their progenitors. AEC may lack Major Histocompatibility (MHC) antigens implying a minimal rejection after transplantation. Thus, we investigated if a) AEC express markers characteristic of pluripotent cells, b) are clonogenic and form teratomas, c) differentiate into cells derived from the germ layers and d) changes in MHC antigens prior to after differentiation. **Methods:** AEC from term fetal membranes were used (n=15). a) mRNA/protein expression of pluripotent cell markers was assessed by RT-PCR and immunocytochemistry, respectively. b) AEC (20 cells/cm^2) treated with or without growth factors were tested for clonal expansion. SCID mice testes injected with AEC were monitored for teratoma formation. c) AEC were cultured with supplements to induce differentiation. d) Percentage of cells presenting MHC Class IA and II antigens assessed by FACS. **Results:** a) Native, term AEC expressed mRNA and/or protein of pluripotent stem cell markers including Oct-4, Sox-2, Nanog. b) AEC formed large clonal colonies. EGF+FGF (10ng/ml each) stimulated proliferation significantly compared to controls in DMEM:F12 or cells stimulated with EGF, Activin A or FGF alone (p<0.0001). Teratomas were absent in SCID mice testes 10weeks post injection. c) Native AEC differentiated into hepatic, pancreatic (endodermal), myocytic, adipocytic, cardiomyocytic, osteocytic (mesodermal), neural and glial (neuroectodermal) cells as shown by phenotypic, electron microscopic and/or mRNA, protein, FACS analyses for markers specific for each cell type. d) Less than 2% of native undifferentiated AEC contained MHC Class IA and II antigens. Upon differentiation into cardiomyocytic, hepatic or pancreatic cells MHC Class II remained suppressed (<14%). **Conclusions:** Term AEC display the properties of pluripotent stem cells but importantly, do not form teratomas, unlike pluripotent embryonic stem cells. Suppression of MHC Class II antigens following differentiation suggests that rejection after transplantion may be minimal. Term amnion, a tissue that is normally discarded at birth, may be a novel, less controversial, highly abundant and easily accessible source of stem cells for regenerative and reparative medicine.

555

Role of Stem Cells in Estrogen Induced Endometrial Regeneration. Caroline E Gargett,* Rachel WS Chan. *Centre for Women's Health Research, Dept. Obstetrics & Gynaecology, Monash Institute of Medical Research, Monash University, Melbourne, Victoria, Australia.*

Objectives: Rare candidate endometrial epithelial and stromal stem/progenitor cells have been identified in mouse endometrium as label retaining cells (LRC).[1] The LRC technique identifies adult stem cells on the basis of their relatively infrequent rate of cell division compared to their more mature progeny. Epithelial LRC are ERα negative and are located in the luminal epithelium, while 16% of stromal LRC express ERα and are located perivascularly at the endometrial myometrial junction and beneath the luminal epithelium. The aim of this study was to determine whether endometrial epithelial and stromal LRC proliferate in response to estrogen to initiate estrogen-induced endometrial regeneration.

Methods: Postnatal day 3 female C57B/6J mice received multiple injections of BrdU (50 μg/g) for 3 days followed by a chase period of 4 or 8 weeks, then ovariectomised to regress the endometrium for 7 days, after which a single 17β-estradiol injection (5 ng/g) was given to stimulate endometrial regeneration. Uteri were harvested 0, 2, 8, 16, 24 and 48 hours later. Sections were double immunostained with BrdU and Ki-67 to detect LRC and proliferating cells respectively, and examined by confocal microscopy.

Results: Ki-67 immunoreactivity was first observed in endometrial cells 8 hours post estrogen treatment, almost exclusively in epithelial and stromal LRC. All epithelial LRC commenced proliferation at 8 hours and the percentage of $BrdU^{+}Ki\text{-}67^{+}$ cells remained constant over 48 hours, while the percentage of proliferating epithelial cells ($Ki\text{-}67^{+}$) progressively increased between 8 and 48 hours post estrogen. In contrast, only 12% of stromal LRC commenced proliferation at 8 hours and the percentage of $BrdU^{+}Ki\text{-}67^{+}$ stromal cells continued to increase over the next 40 hours. Stromal cell proliferation also gradually increased between 8 and 48 hours.

Conclusions: This study demonstrates that estrogen has different effects on endometrial epithelial and stromal LRC, and suggests that candidate epithelial stem cells initiate the cyclical growth of endometrial epithelium, while stromal LRC are gradually recruited into cell cycle and have a lesser role in regenerating endometrium. It also suggests that the proliferative effects of estrogen on epithelial LRC is indirect and is likely mediated via estrogen action on neighboring ERα-expressing niche cells.

[1]Chan RWS, Gargett CE (2006) Stem Cells 24:1529-38.

556

Down-Regulation of IGF-1R Signaling in Trophectoderm Stem Cells Is Reversed by Stimulation of AMPK Via AICAR or Phenformin – A Model for Maternal Hyperinsulinemia and Pregnancy Loss. Erica Louden, Maggie Chi, Kelle Moley.* *OB/GYN, Washington University School of Medicine, St. Louis, MO, USA.*

Objective: Trophoblast Stem cells (TS) are derived from trophectoderm, one of the earliest cell types to be formed in the blastocyst stage embryo. The trophectoderm and later the trophoblast are essential for survival of the embryo because it mediates implantation and ultimately becomes the placenta. During embryo development a number of growth factors influence the maternal environment. At the blastocyst stage high concentrations of IGF-1 or insulin, characteristic of maternal hyperinsulinemia, lead to extensive apoptosis and a significant down-regulation of IGF-1R. The down-regulation of IGF-1R at this developmental stage, as a result of elevated IGF-1 or insulin, is responsible for decreased glucose uptake and increased apoptosis that results in poor pregnancy outcomes in mice. Moreover, this process may account for the increase in pregnancy loss in the insulin resistant patient population. Our objective was to discover the mechanisms responsible for the increased apoptosis, decreased glucose transport and poor pregnancy outcomes using a TS cell model. In addition, we hypothesize that dysfunction of AMPK activity may play a role in this process.

Method and Results: We found that down-regulating the expression of IGF-1R with siRNA in TS cells resulted in decreased expression of P-AMPK and P-ACC (n=6). The efficiency of transfection with the siRNA in reducing IGF-1R content was confirmed by Western blotting. Furthermore, employing small interference RNA directed against IGF-1R caused a 48% decrease in glucose uptake that was shown to be insulin dependent and increased apoptosis by 40% (n=3). The effect of down-regulation of IGF-1R on P-AMPK, P-ACC, apoptosis and glucose uptake was reversed with treatment of the AMPK-activator, 5'-aminoimidazole-4carboxamide-1-β-D-ribofuranoside (AICAR) (250μM for 2 hours) and/or Phenformin (5mM for 2 hours) (n=3). AICAR stimulation of AMPK increased insulin-stimulated glucose up-take by over a 100% ($p<0.01$). Inhibition of AMPK activity by compound C (125μM) negated the anti-apoptotic effects of AICAR.

Conclusion: These results suggest that down regulation of the IGF-1R leads to decreased AMPK activity. This kinase activity appears to be necessary and sufficient for the stimulation of glucose transport and maintenance of cellular survival in TS cells experiencing an apoptotic response to hyperinsulinemia.

557

Peripubertal Hyperinsulinemia Up-Regulates Phosphatidylinositol 3-Kinase/Protein Kinase B Pathway in Rat Ovaries. Shilla Chakrabarty, Manubai Nagamani.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective: Polycystic ovary syndrome (PCOS) is associated with insulin resistance and hyperinsulinemia. PCOS patients with hyperinsulinemia have profound peripheral insulin resistance, but retain sensitivity to insulin in the ovary. The cause of this paradox is little known. Because insulin and insulin receptors are widely present in the ovary, and insulin modulates ovulation and ovarian steroidogenesis, we evaluated the regulation of insulin signaling pathways in ovaries of rats with peripubertal hyperinsulinemia.

Methods: We induced experimental hyperinsulinemia in newly weaned female rats by infusing insulin (0.14 IU/day) for 4 weeks via subcutaneously implanted Alzet minipumps. We infused Control animals with normal saline. At autopsy, (rat age 56 days), the ovaries were quickly removed, and snap frozen in liquid nitrogen for protein extraction, or fixed in Bouin's fluid for histological and immunohistochemical studies. Protein extracts from whole ovaries were used to study the effects of hyperinsulinemia on the Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3-kinase) pathway necessary for initiation of glucose transport, and on Mitogen Activated Protein Kinase (MAPK/ERK1/2) pathway used for producing the mitogenic effects of insulin.

Results: In comparison to Control, peripubertal hyperinsulinemia remarkably up-regulated total protein kinase B (Akt), and enhanced Akt serine and theronine phosphorylation in rat ovaries. Phosphorylation, and therefore inactivation of glycogen synthase kinase-3 (GSK-3) was also markedly increased, suggesting that glycogen synthesis was inhibited by hyperinsulinemia. Phosphorylation of Raf, however, was not affected, suggesting that hyperinsulinemia did not modulate the Ras/Ref/MEK/ERK pathway in the ovaries. However, the activity of Phosphatase and tensin homolog (PTEN) protein, a lipid phosphatase and a major negative regulator of the PI3 kinase/Akt signaling pathway, was markedly down regulated by hyperinsulinemia.

Conclusion: These findings, along with our observations of estrus acyclicity, hyperandrogenism, and ovarian changes similar to those seen in women with PCOS, namely, thecal and interfollicular stromal proliferation, and premature lutenization of ovarian follicles, suggest that the ovarian PI-3 kinase pathway may be involved in the development of PCO-related ovarian dysfunction in peripubertal hyperinsulinemia.

558

A SNP in the Liver Kinase Gene LKB1 Is Associated with Failure To Ovulate on Metformin Therapy. Richard S Legro,*[1] Sandra A Carson,*[1] Michael P Diamond,*[1] William D Schlaff,*[1] Bruce R Carr,*[1] Michael P Steinkampf,*[1] Christos Coutifaris,*[1] Peter G McGovern,*[1] Linda C Giudice,*[1] Phyllis C Leppert,*[1] Nicholas A Cataldo,*[1] Gabriel Gosman,[1] John E Nestler,[1] Kathy G Ewens,[2] Richard S Spieman,[2] Huiman X Barnhart,[1] Evan R Myers.[1] *[1]Reproductive Medicine Network; [2]National Cooperative Program in Infertility Research.*

a) Objective: We hypothesized that genetic variation in the estrogen receptor, in LKB1 involved in hepatic glucose homeostasis and metformin action, and CYP genes (2D6/2C9) involved in steroid metabolism would predict ovulatory response.

b) Methods: We examined polymorphisms in these genes related to drug action in a substudy of women (N = 311) who participated in our multi-center trial, Pregnancy in Polycystic Ovary Syndrome, of clomiphene (CC), metformin(MET), and the combination in infertile women with PCOS. Generalized estimating equations were used for analysis of ovulation rate to account for correlation of multiple ovulation cycles within a subject. We used stepwise regression with an alpha level of 0.1 in selecting baseline risk factors for ovulation.

c) Results: We found no significant effect between SNPs from the ER or CYP genes and ovulation, but we noted a significant negative effect of genotypes containing the 2 allele from a SNP in the LKB1 gene in the MET arm, which approached significance in the same trend in the CC arm. Other factors in the

predictive model for ovulation were decreasing BMI (BMI < 30: OR[95% CI] 2.36[1.65,3.36] vs BMI > 35), a lower baseline Free Androgen Index (FAI < 10: 1.59 [1.17,2.18] vs FAI > 10), a shorter duration of prior infertility (< 18 mos: 1.63[1.20,2.21] vs > 18 mos), and a history of a prior pregnancy loss (1.77[1.26,2.49]vs none). We noted no racial or ethnic effects.

d) Conclusion: Though the treatment related differences remain puzzling, this is the first evidence that genetic variation in a MET response gene may be associated with altered efficacy. These findings may have substantial impact on the selection of subjects for MET use in both PCOS and type 2 DM.

Association of LKB1 SNP with Ovulation by Treatment Arm

	Genotype	Ovulation per cycle Odds ratio (95% CI)	P Value
Clomiphene	3/3	1.0	
	2/2	0.53 [0.30,0.95]	0.071
	2/3	0.60 [0.35,1.02]	0.12
Metformin	3/3	1.0	
	2/2	0.30 [0.14,0.66]	0.011
	2/3	0.30 [0.16,0.56]	0.002
Combined	3/3	1.0	
	2/2	0.98 [0.48,2.00]	0.97
	2/3	0.72 [0.40,1.31]	0.37

559

Hyperandrogenism Is Related to Reactive Oxygen Species (ROS) Generation from Pre-Activated Leukocytes in Polycystic Ovary Syndrome (PCOS). Frank Gonzalez,[*1] Neal S Rote,[*2] Judi Minium,[2] John P Kirwan.[3] *[1]Obstetrics and Gynecology, Mayo Clinic College of Medicine, Rochester, MN, USA; [2]Reproductive Biology, Case Western University School of Medicine, Cleveland, OH, USA; [3]Gastroenterology and PathoBiology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA.*

Objective: Women with PCOS exhibit increased leukocyte sensitivity characterized by increased reactive oxygen species (ROS) generation in response to a dietary carbohydrate trigger. We evaluated the status of leukocytic ROS generation in the fasting state in women with PCOS compared to ovulatory controls, and the relationship between fasting ROS generation and circulating LH and androgens.

Methods: Fourteen women with PCOS (7 lean, 7 obese) between ages 18-40 yrs, diagnosed on the basis of oligo- or amenorrhea and hyperandrogenemia, and 12 ovulatory controls (6 lean, 6 obese) of similar age were selected for study. Subjects with diabetes, inflammatory illnesses or other endocrinopathies were excluded. ROS generation was measured by chemiluminescence in leukocytes isolated from fasting blood samples. Serum LH and androgens were measured by RIA. Insulin sensitivity was derived by IS_{OGTT}.

Results: There were no significant differences in age and body mass index between the women with PCOS and weight-matched controls. Lean women with PCOS exhibited a significantly lower IS_{OGTT} compared to lean controls (5.4±0.4 vs. 8.0±1.3, $p<0.03$). As expected, a significantly ($p<0.002$) lower IS_{OGTT} was also evident in obese women with PCOS (3.5±0.6) and obese controls (3.9±0.7) compared to lean controls. ROS generation from mononuclear cells (MNC) was significantly greater in women with PCOS compared to weight-matched controls (Lean, 847±141 vs. 394±74 mV, $p<0.02$; Obese, 1115±149 vs. 378±92 mV, $p<0.0005$). ROS generation from polymorphonuclear cells (PMN) was significantly greater in obese women with PCOS compared to obese controls (2511±474 mV, $p<0.0004$). Leukocytic log ROS generation was positively correlated with serum levels of LH (MNC, $r=0.56$; $p<0.004$), Testosterone (MNC, $r=0.46$; $p<0.02$; PMN, $r=0.45$; $p<0.03$), Androstenedione (MNC, $r=0.66$; $p<0.0004$; PMN, $r=0.66$; $p<0.0006$) and DHEA-S (MNC, $r=0.53$; $p<0.006$; PMN, $r=0.50$; $p<0.02$).

Conclusions: These preliminary data indicate that in PCOS, increased leukocyte sensitivity is due to pre-activation as evidenced by increased fasting ROS generation independent of obesity. Hyperandrogenism in PCOS may promote this phenomenon.

560

Epigenetic Regulation of Decidualization of Human Endometrial Stromal Cells. Philip C Logan,[1,2] Fahimeh Rahnama,[1,2] Peter E Lobie,[1,2] Murray D Mitchell.[*1,2] *[1]The Liggins Institute, University of Auckland, Auckland, New Zealand; [2]National Research Centre for Growth and Development, C/-The Liggins Institute, Auckland, New Zealand.*

The decidualization of endometrial stromal cells to decidual cells is essential for a week old embryo to successfully implant into the uterine endometrium, form a placenta and for the pregnancy to be maintained. To induce decidualization we used the epigenetic mechanism of DNA methylation. DNA methylation silences genes but if the methylation of a gene is inhibited then that gene will be up-regulated.

Objective To determine whether alteration in DNA methylation can result in decidualization of human endometrial stromal cells.

Methods The methylation inhibitor, AZA (5'-aza-2'-deoxycytidine) was given as a 15uM treatment to a human endometrial stromal cell-line, HESC. As a control the HESC cells were given MPA-mix (medroxyprogesterone acetate /estradiol /dibutyryl cAMP) the ovarian hormone initiators and sustainers of decidualization. RT-PCR was used to evaluate the changes in gene expression specifically for PRL, IGFBP-1, FKHR, TIMP3, CNR1.

Results When HESC cells were treated with AZA the two decidualization indicators showed decidualization, i.e. the change in morphology and the change in gene expression. AZA altered the morphology of stromal cells from fibroblast-like to rounder, decidualized cells, and when these cells were compared with the morphology of stromal cells treated with MPA-mix both morphologies appeared similar. During decidualization the marker genes prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) are up-regulated (along with many other genes) from virtually no expression. Semi-quantitative RT-PCR showed that the prolactin and IGFBP-1 genes were up-regulated many fold by 15uM AZA treatment over twelve days. When 15uM AZA was combined with the MPA-mix and given to HESC cells for twelve days there was a 1.5 - 2 fold increase in expression in five decidualization marker genes (PRL, IGFBP-1, FKHR, TIMP3, CNR1) compared with the MPA-mix only treatment.

Conclusion Both the indicators of decidualization, i.e. the change in morphology and the up-regulation of prolactin and IGFBP-1, show that decidualization is epigenetically regulated.

561

Fetoplacental Abnormalities Quantified by Micro-Computed Tomography in a Murine Model of Pre-Pregnancy Smoking. Monique Y Rennie,[1,3,5] Jacqui Detmar,[2,5] Kathie J Whiteley,[2] Andrea Jurisicova,[2,5] S Lee Adamson,[*2,4,5] John G Sled.[1,3,5] *[1]MICe, Hospital for Sick Children; [2]SLRI, Mount Sinai Hospital; [3]Dept of Medical Biophysics; [4]Dept of Ob/Gyn and Physiology; [5]University of Toronto, Toronto, ON, Canada.*

Background: Smoking-related fetal growth restriction may be due in part to polycyclic aromatic hydrocarbon (PAH) exposure. Prepregnancy injections of two PAHs, benzo(a)pyrene (BaP) and dimethylbenzanthracene (DMBA), cause IUGR in mice and histological changes in the placental labyrinth. In this study we assessed fetoplacental vascularity in offspring of PAH-exposed mice using micro-computed tomography (microCT), a high resolution 3D X-ray imaging technique.

Methods: Female mice were injected once a week during weeks 1-3 and 7-9 with either a BaP-DMBA mix (2mg/kg) or corn oil vehicle (4 mothers/grp). One week post last injection females were mated, with no further injections given during pregnancy. The fetoplacental vasculature was perfused with radio-opaque contrast agent at day 15.5 of gestation and scanned using microCT. Data reconstruction produced 3D images and generated geometric models of the lumen surface, excluding capillaries.

Results: Fetuses from treated mice were growth restricted 22% by weight, yet placental weight was unchanged. Significant decreases were observed in umbilical artery and vein diameters (14% and 18%), total observed arterial vascular surface area (19%) and total observed arterial vascular volume (20%).

		BaP-DMBA (N=8)	Vehicle (N=9)	p-value
Fetal weight (g)		0.34 ± 0.01	0.44 ± 0.02	>0.001
Placental weight (g)		0.152 ± 0.004	0.152 ± 0.004	NS
Umbilical Diameter (mm)	Arterial	0.45 ± 0.01	0.52 ± 0.01	>0.001
	Venous	0.41 ± 0.02	0.50 ± 0.01	>0.001
Vascular surface area (mm^2)	Arterial	77.6 ± 2.9	95.5 ± 4.9	0.01
	Venous	55.9 ± 3.3	52.7 ± 2.2	NS
Vascular volume (mm^3)	Arterial	2.74 ± 0.11	3.42 ± 0.18	0.01
	Venous	2.45 ± 0.14	2.29 ± 0.11	NS

Values shown as mean ± SEM where N is number of placentas. NS = Not significant

Conclusions: Changes in fetoplacental arterial and venous, but not capillary, vessels caused by pre-pregnancy PAH exposure were quantifiable by microCT. Reduced umbilical diameters suggest reduced fetoplacental perfusion. Thus, fetal growth restriction due to pre-pregnancy PAH exposure may be due in part to impaired placental function.

FRIDAY

562

The One-Two Punch by alpha-C-Galactosylceramide Promotes Ovarian Tumor Immunity. Pankaj K Singhal,[1,2] Axel Mischo,[2] Qingsheng Li,[2] Cheryl Eppolito,[2] Moriya Tsuji,[3] Shashikant Lele,[1] Kunle Odunsi,[*1,2] Protul Shrikant.[2] *[1]Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA; [2]Immunology, Roswell Park Cancer Institute, Buffalo, NY, USA; [3]Medical and Molecular Parasitology, New York University School of Medicine, NY, NY, USA.*

Introduction

The novel glycolipid α-C-galactosylceramide (α-C-GalCer) has selective ability to activate DC, NK and NKT cells to produce IFN-γ and IL-12 leading to type-I responses. We have shown that a high ratio of $CD8^+$ effector T cells and $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) predicts improved prognosis in epithelial ovarian cancer (EOC) patients *(Sato et.al PNAS)*.

Objectives

We tested the ability of α-C-GalCer in regulating host adaptive immune responses and achieving durable tumor immunity.

Methods

In our murine OVA expressing syngeneic ovarian tumor model ($H\text{-}2K^b$+ MOSEC-OVA), naïve TCR transgenic $CD8^+$ (OT-1) and $CD4^+$ (OT-II) T cells specific for OVA were adoptively transferred (2×10^6) in age matched tumor (20×10^6 MOSEC-OVA) bearing female C57BL6, $IL12^{-/-}$ and $CD1d^{-/-}$ mice. Each group was vaccinated (1ug α-C GalCer + 5ug OVA protein) and harvested at various time points with findings confirmed in repeat experiments.

The relative numbers of $CD4^+$, $CD8^+$ and Treg T cells were enumerated in spleen, lymph nodes and peritoneal cavity. Effector function was assessed by *in-vivo* CTL and *in-vitro* intracytoplasmic cytokines expression. Protection and survival from tumor challenge were plotted using Kaplan-Meier and analyzed by log-rank.

Results

The α-C-GalCer efficiently enhances the clonal expansion of OT-1, OT-II and endogenous $H\text{-}K^b$/SIINFEKL specific $CD8^+$ T cells (>100 fold, $P < 0.0001$).

The induced $CD8^+$ T cell clonal expansion was largely due to proliferation (CFSE dilution) and also attributable to increased survival (decreased T cell apoptosis).

This expansion was associated with robust $CD8^+$ T cell differentiation and maturation due to endogenous IL-12 production and was alsodependent on CD1d presentation. The α-C-GalCer induced IL-12 production also reduced the tumor associated Tregs in the peritoneal cavity ($P < 0.004$) that correlated with increased survival of tumor bearing host in the immunized group ($P < 0.003$).

Conclusions

α-C-GalCer promotes antigen specific clonal expansion and effector differentiation of $CD8^+$ and $CD4^+$ T cells. It also reduces frequency of Tregs in EOC microenvironment with improved survival providing a strong rationale for its use as an adjuvant EOC immunotherapy.

563

Heme Oxygenase-1 Is a Negative Regulator of Soluble Flt-1 and Soluble Endoglin. Melissa Cudmore, Shakil Ahmad, Bahjat Al-Ani, Kunal Chudasama, Takeshi Fujisawa, Peter Hewett, Asif Ahmed. (SPON: Stephen K Smith). *Reproductive and Vascular Biology, Centre for Cardiovascular Sciences, Institute of Biomedical Research, The Medical School, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

Background/Objective: Preeclampsia is characterised by hypertension, proteinuria, and dysregulated angiogenesis. Soluble Flt-1 (sFlt-1) and endoglin (sEng) are elevated in preeclampsia and their co-administration to rats elicits severe preeclampsia-like symptoms. Heme oxygenase-1 (HO-1) and its metabolite, carbon monoxide (CO) exerts protective effects in several organs against oxidative stimuli. As HO-1 is down regulated in preeclampsia we investigated the role of HO-1 in the regulation of sFlt-1 and sEng.

Results: Adenoviral overexpression of vascular endothelial growth factor (VEGF) induced a three-fold increase plasma sFlt-1 levels in mice. VEGF-mediated sFlt release required VEGF receptor-2 activation as SU1498 blocked its production. Endothelial cells overexpressing HO-1 or pre-treated with carbon monoxide (CO)-releasing molecule (CORM-2) or CO gas decreased the basal and VEGF- and interferon-γ (INF-γ) induced sFlt-1 release; CO inhibited VEGF-induced sFlt-1 by inhibiting VEGFR-2 phosphorylation. Ttreatment of villous explants or endothelial cells with the HO inhibitor, tin protoporphyrin-IX potentiated VEGF and INF-γ induced sFlt-1 expression, as did siRNA knockdown of HO-1. Consistent with these findings, levels of sFlt-1 in plasma and tissue lysates of HO-1-deficient mice were significantly higher than wild-type mice. sEng release was greatly elevated in preeclamptic placental explants and administration of AdHO-1 significantly decreased the basal, IFN-γ and TNF-α induced sEng from endothelial cells while siRNA to HO-1 increased basal sEng and potentiated cytokine-induced production. Interestingly, Vitamin C and E had no effect on sFlt-1 or sEng release or HO-1 protein expression consistent their lack of efficacy in preeclampsia, whereas, statins inhibited sFlt-1 release from endothelial cells and explants and upregulated HO-1.

Conclusion: This study conclusively establishes HO-1/CO pathway as a negative regulator of cytokine-induced sFlt-1 and sEng release. As elevated levels of sFlt-1 and sEng are associated with the clinical symptoms of preeclampsia, these findings provide compelling evidence for a protective role of HO-1 in pregnancy and identify a novel target for the treatment of preeclampsia.

564

Fumonisin Mycotoxin Contamination of Corn-Based Foods Consumed by Potentially Pregnant Women in Southern California. Natalia J Dvorak,[1] Ron T Riley,[2] Mary Harris,[3] James A McGregor.[*1] *[1]Obstetrics and Gynecology, Keck School of Medicine at USC, Los Angeles, CA, USA; [2]Toxocology and Mycotoxin Research Unit, USDA/ARS/SAA, Athens, GA, USA; [3]Human Nutrition, Colorado State University, Fort Collins, CO, USA.*

Objective: Evaluate corn-based staple foods (tortillas and masa flour) for fumonisin mycotoxin (FB) contamination.

Background: *Fusarium* spps are fungi that infect corn plants and grain stocks worldwide. *Fusarium* spps produce mycotoxins (FB1, FB2, others), which interfere with mammalian folic acid receptor (FAr) activity. Consumption of fumonisin mycotoxin contaminated food is linked to increased risk of neural tube defects (NTDs). Fumonisin contamination may be a common but underappreciated cause of FA insufficiency and subsequent NTDs. Hispanic women may be at increased risk of folic acid dietary deficiency because of FB consumption as well as having an increased frequency of the MTHFR 677T polymorphism, low folic acid food intake, and lack of multivitamin supplementation.

Methods: We obtained 38 samples of corn-based tortillas and masa flour in geographically and ethnically diverse grocery stores and restaurants in Los Angeles, San Diego, and Tijuana, Mexico. Retail sources were diverse and not limited to Hispanic neighborhoods. HPLC analysis for fumonisins and related substances was performed at the USDA Toxicology and Mycotoxin Research Unit in Athens, Georgia.

Results: Testing demonstrated the presence of FB1, 2, 3 and the hydrolyzed form of FB1 in 38/38 samples. Average FB1 levels were 160 ng/g +/- 204ng/g. Average total fumonisin levels were 414ng/g +/- 524 ng/g. Levels of fumonisins differed by geographic site (highest in Hispanic neighborhoods and Tijuana, Mexico).

Conclusion: Fumonisin mycotoxin contamination of corn-based staple foods available in diverse southern California neighborhoods is common. FB mycotoxins are linked to both outbreak and endemic occurrences of NTDs among Hispanic-Americans and other populations. With the levels of contamination found, a 60kg woman could exceed the WHO recommendations (for non-pregnant adults) by eating 2-4 tortillas daily. Fumonisin food contamination may constitute a preventable cause of NTDs in populations at risk. Calculations of the attributable fractions of NTDs caused by mycotoxin contamination, in conjunction with other factors, may be used to inform public and personal health strategies to reduce risks of NTDs and other sequelae of folic acid insufficiency.

565

Congenital Heart Defects and Maternal Dietary Vitamin A and E Intake. Huberdina PM Smedts,[1] Jeanne H de Vries,[2] Maryam Rakhshandehroo,[1] Mark F Wildhagen,[1] Anna C Verkleij-Hagoort,[1] Eric AP Steegers,[*1] Regine PM Steegers-Theunissen.[1,3,4,5] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Human Nutrition and Epidemiology, Wageningen University, Wageningen, Netherlands; [3]Epidemiology and Biostatistics; [4]Pediatric Cardiology; [5]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

BACKGROUND: Maternal hyperhomocysteinemia is a risk factor for a child with a congenital heart defect (CHD). This may indicate an increased intrauterine exposure to oxidative stress or reduced antioxidant defence or both during embryonic cardiac development.

OBJECTIVE: To investigate associations between the maternal dietary intake of the antioxidants vitamin A and E, maternal hyperhomocysteinemia and CHD risk in the offspring.

METHODS:A case-control family study was conducted in 192 mothers of a child with CHD and in 216 mothers of a healthy child. Mothers filled out a general and food frequency questionnaire at 17 months post partum as a proxy of the periconceptional dietary intake. Pregnant or lactating mothers, or those who changed their diet compared to the periconceptional diet were excluded for analysis. Intakes are presented as geometric means (P5-P95). Logistic regression analyses were performed and odds ratios (OR) with 95% confidence intervals (95% CI) were calculated.
RESULTS: The dietary intake of vitamin A in case- and control mothers of 486 (169-1381) and 480 (192-1422) µg/day, respectively, were comparable and not associated with CHD risk. Control-mothers reported a lower dietary intake of vitamin E compared to case-mothers, 11.8 (7.3-19.1) and 12.5 (7.8-19.2) mg/day, respectively (p=0.02). The dietary intakes of vitamin A and E were not significantly different within and between hyper- and normohomocysteinemic case- and control-mothers. A significant trend was shown towards a risk reduction by lower intakes of vitamin E (p=0.02). Periconceptional folic acid or multivitamin supplemented mothers who had a low dietary intake of vitamin E (9.3-11.0 mg/day) demonstrated a nearly 60% reduced CHD risk, OR 0.41(0.18-0.93).
CONCLUSIONS:For the first time a teratogenic effect of a maternal dietary vitamin E intake in the upper level is suggested. Moreover, a low dietary vitamin E intake in addition to vitamin supplement use reduces CHD risk. Studies should be performed to investigate the beneficial and teratogenic effects of natural and synthetic antioxidants on reproductive outcome.

566

Extreme Interpregnancy Intervals Are Associated with Adverse VBAC Outcomes. David Stamilio,[1] Emily DeFranco,[1] Emmanuelle Pare,[2] Anthony Odibo,[1] Jeffrey Peipert,[*1] Jenifer Allsworth,[1] Erika Stevens,[1] George Macones.[*1] *[1]Ob/Gyn, Washington University in St. Louis, St. Louis, MO, USA; [2]Ob/Gyn, University of Pennsylvania, Philadelphia, PA, USA.*
Objective: To investigate whether a short or long interpregnancy interval (IPI) is associated with major maternal morbidity in women who attempt vaginal birth after cesarean (VBAC).
Methods: We performed a multicenter record-based retrospective cohort study of pregnant women with at least one prior cesarean between 1996 and 2000. Trained research staff collected data on demographics, medical and obstetric history, prenatal and intrapartum course, delivery and maternal outcomes. This planned secondary analysis included all patients that attempted VBAC. We performed bivariate and multivariable logistic regression analyses to evaluate the association between long or short IPI and four primary maternal outcomes, including: (1) uterine rupture, (2) a composite major morbidity variable (including rupture, bladder or bowel injury, and uterine artery laceration), (3) blood transfusion and (4) postpartum fever. We evaluated short IPI with cutoffs at <6, <12 and <18 months between prior delivery and conception, and defined long IPI as >60 months.
Results: Of 13331 VBAC attempts, 128 cases (0.9%) of uterine rupture occurred. There were 286 (2.2%), 1395 (10.5%), 3136 (23.5%) and 2631 (19.7%) women with an IPI <6, <12, <18 and >60 months, respectively. An IPI <6 months was associated with an increased risk for uterine rupture, major morbidity, and blood transfusion. Risks were also increased with an IPI<12 months, but odds ratios were smaller and statistically nonsignificant. Uterine rupture risk was highest in women with a short IPI and gestational diabetes (GDM), but there is low precision for this finding due to the small case number. A long IPI was associated with an increased risk of fever (odds ratio 1.2, 95%CI 1.1-1.4).
Conclusions: Short IPI is a risk factor for uterine rupture and other major morbid events in VBAC candidates. The length of IPI should be considered when counseling women about the risks and benefits of VBAC. Avoiding a short IPI may reduce risk associated with VBAC.

IPI < 6 months in VBAC patients	Short IPI n/d (%)	Normal IPI n/d (%)	Adjusted* Odds Ratio	95% Confidence Interval
Uterine rupture	8/286 (2.8)	118/13045 (0.9)	2.66	1.21-5.82
Composite morbidity	12/286 (4.2)	282/13045 (2.2)	1.95	1.04-3.65
Blood transfusion	7/286 (2.4)	86/13045 (0.6)	3.14	1.42-6.95
Uterine rupture in GDM patients			Unadjusted Relative Risk	
IPI< 6 months	1/6 (16.7)	5/588 (0.9)	19.60	2.68-143.49
IPI<12 months	3/61 (4.9)	3/533 (0.6)	8.73	1.80-42.35
IPI<18 months	4/119 (3.4)	2/475 (0.4)	7.98	1.48-43.07

*Models include all significant confounding variables & effect modifiers.

567

A Randomised Controlled Trial of Cervical Scanning vs History To Determine Cerclage in High Risk Women (CIRCLE Trial). Rachael Simcox,[1] Phillip Bennett,[*2] TG Teoh,[3] Andrew H Shennan.[1] *[1]Division of Reproduction and Development, King's College London, London, United Kingdom; [2]Parturition Research Group, IRDB, Imperial College London, London, United Kingdom; [3]Obstetrics and Gynaecology, Imperial College London, London, United Kingdom.*
Objective
To determine the effects of cervical scanning and cervical stitches in preventing preterm birth (PTB) in women at high risk.
Methods
A randomised controlled trial recruited 250 women at high risk of PTB from 8 centres in the UK. Randomisation was stratified for gestational age at previous PTB. 126 women were randomised to 'traditional' management whereby a cervical suture was offered based on a history of cervical insufficiency. 119 women were randomised to 'scanning' management with a suture inserted if the cervical length was ≤20mm measured by transvaginal ultrasound prior to 24 weeks. Primary outcome was spontaneous PTB<34 weeks.
Results
Data on 245 women available at submission is reported. Past obstetric history was comparable; with 85 v 80 previous PTB and 79 v 79 previous second trimester losses in the traditional management group v scanning group respectively. In the 119 women allocated to scanning, 39 (33%) with a cervix that shortened to ≤20mm received an ultrasound indicated suture. This compared with 25 of 126 (20%) women in the traditional arm, who received an elective suture based on history alone (RR 1.7 95% CI 1.1-2.6). There was no difference in the rate of delivery <34 weeks between the two groups (ultrasound group 15/119 v traditional group 16/126 (RR 1.0 95% CI 0.5-2.0). All interventions were numerically more likely in women scanned including hospital admissions, bed rest, steroid administration, and tocolysis, and significantly so for progesterone (RR 1.6 CI 1.1-2.3).
Conclusion
In women at high risk of PTB, offering ultrasound surveillance of cervical length and cerclage for those with a short cervix does not alter the risk of PTB prior to 34 weeks when compared with offering a suture electively based on a history suggestive of cervical insufficiency. Furthermore, those women who are offered ultrasound surveillance are almost twice as likely to be offered a suture. Other interventions may also be more likely to be offered to the group whom are scanned with no translation to an improvement in gestational age at delivery. These data do not suggest any benefit in replacing ultrasound surveillance with historical indications for suture placement.
This work was funded by Tommy's the baby charity.

568

Paradoxical Upregulation of Placental Leptin Expression in Gestationally Food Restricted Rat Dams. Andrea Jelks, Guang Han, Michael G Ross,* Mina Desai. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*
BACKGROUND: Leptin, a hormone produced by adipose tissue normally acts as a satiety signal in the hypothalamus. Leptin is also produced by the placenta and is thought to serve as a placental and fetal growth factor. Maternal food restriction (MFR) during pregnancy results in reduced maternal and newborn plasma leptin levels. To assess the etiology of reduced leptin, we sought to determine leptin expression in the adipose tissue and placenta of MFR dams.
METHODS: Control dams (n=6) received ad libitum food whereas study dams were 50% food restricted (MFR, n=6) from pregnancy days 10 to 16. At E16, dams were sacrificed and maternal retroperitoneal adipose tissue and placentas were collected. Adipose tissue leptin protein expression was determined by Western Blot, and placental leptin mRNA expression was determined by real-time RT-PCR, normalized to β-actin. Plasma leptin levels were determined by radioimmunoassay. Results are reported as mean ± SE.
RESULTS: At E16, MFR dams weighed significantly less than Control dams (255±6 vs 289±8 g, p<0.01) and had significantly decreased plasma leptin levels (0.71±0.13 vs 1.39± 018 ng/ml, p<0.05). Maternal adipose tissue leptin expression was significantly reduced in MFR as compared to Control dams (577±100 vs 1208±119 AU, p<0.01). Conversely, placental leptin mRNA expression was significantly elevated in MFR pregnancies (2.4±0.06 fold, p<0.01).
CONCLUSION: MFR rat dams demonstrate decreased plasma leptin levels primarily due to decreased leptin production by adipose tissue. Paradoxically, placental leptin expression is increased in response to MFR, potentially serving as a compensatory mechanism to augment feto-placental growth during a period of severe nutrient stress.

569

Determinants of Blood Oxygenation during Pregnancy in Andean and European Residents of High Altitude. Marco Vargas,[1] Enrique Vargas,[1] Colleen G Julian,[3,4] J Fernando Armaza,[1] Armando Rodriguez,[1] Wilma Tellez,[1] Susan Niermeyer,[3] Megan J Wilson,[3,4] Esteban Parra,[2] Mark Shriver,[2] Lorna G Moore.*[3,4] *[1]Instituto Boliviano de Biologia de Altura, Universidad Mayor de San Andres, La Paz, Bolivia; [2]Genetics Laboratory, Department of Anthropology, Pennsylvania State University, State College, PA, USA; [3]Altitude Research Center, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA; [4]Department of Health and Behavioral Sciences, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA.*

Objective: High altitude decreases birth weight to a lesser extent in long *vs*. short-resident high-altitude populations. We asked whether greater maternal arterial oxygenation was responsible for these lesser birth weight decreases in long-resident populations. **Methods:** Serial studies were conducted comparing maternal arterial oxygenation in 42 Andean and 26 European residents of La Paz, Bolivia (3600 m) during pregnancy (weeks 20, 30, 36) and 4 mo postpartum. **Results:** Pregnancy raised hypoxic ventilatory sensitivity 3-fold, resting ventilation, and arterial O_2 saturation (S_aO_2) in both groups. Ancestry, as identified using 81 genetic markers, correlated with respiratory pattern such that greater Andean ancestry was associated with higher respiratory frequency and lower tidal volume. Pregnancy increased total and plasma volume ~40% in both groups without changing red cell mass; hence, hemoglobin fell. The hemoglobin decline was compensated for by the rise in ventilation (V_E) and S_aO_2 with the result that arterial O_2 content (C_aO_2) was maintained at or near nonpregnant levels in both groups. Birth weights were similar for the babies born to the Andean and European women but after adjusting for variation in gestational age, maternal height and parity, Andeans weighed 209 gm more than Europeans. Babies with greater ponderal indices were born to Andean women with higher V_E at pregnancy weeks 20, 30 and 36 (R^2 = 0.27, 0.30 and 0.25 respectively, all $p<0.05$). Week 20 V_E also correlated with infant birth weight ($R^2 = 0.15$, $p<0.05$) in the Andean, but not the European women. **Conclusions:** We concluded that while maternal V_E was important, some factor other than higher C_aO_2 was responsible for protecting Andeans from altitude-associated reductions in fetal growth.

570

Adrenomedullin Relaxes Uterine Artery: Mechanisms and Influence of Pregnancy and Female Sex Steroids. Gracious R Ross, Uma Yallampalli, Pandu R Gangula, Luckey C Reed, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Adrenomedullin (ADM) is reported to relax mesenteric artery and these responses are elevated with pregnancy. In this study, we assessed the effect of ADM on uterine artery (UA), its mechanisms and the influence of pregnancy and female sex steroids on the receptors and functional effects of ADM.

OBJECTIVE: To determine: 1) whether ADM relaxes UA; 2) mechanisms of ADM–induced relaxation; 3) whether pregnancy or female sex steroids have any influence on receptors or relaxant effects of ADM.

METHODS: Rats used were either non–pregnant (NP), pregnant (P) on day 18, overiectomized (Ovx), Ovx + implanted with estradiol-17b (E_2 –2 mg/kg, 21 day release) or progesterone (P_4 –20 mg/kg, 21-day release) or placebo pellets (Ovx). UA was used for isometric tension and mRNA analysis of ADM receptor components, receptor activity- modifying protein $(RAMP)_2$ or $RAMP_3$.

RESULTS: ADM relaxed UA from both NP and P rats with pD_2 value of 6.8 ± 0.1 and 6.9 ± 0.1, respectively. But, the E_{max} of ADM was greater in P (70 ± 3%) compared to NP (52 ± 4%) groups. E_2 treatment to Ovx rats increased the sensitivity (pD_2. = 7.2 ± 0.3 vs 6.8 ± 0.3) while E_{max} was not different (79 ± 8% vs 77 ± 14%) and P_4 reduced E_{max} to 38 ± 9%. $RAMP_3$ but not $RAMP_2$ mRNA was increased in pregnancy. E_2 increased mRNA of both $RAMP_2$ and $RAMP_3$ while P_4 had no effect. Combined E_2 and P_4 increased mRNA of $RAMP_3$, but not $RAMP_2$. Mechanisms of ADM–induced relaxation were studied in UA in P rats. ADM_{22-52} shifted the dose–response curve of ADM to the right and E_{max} values were 7 ± 0.1, 60 ± 8% (control) and 6.4 ± 0.4, 42 ± 9%. $CGRP_{8-37}$ was more potent in inhibiting ADM-induced relaxation (E_{max}: 57 ± 10% vs 13 ± 4%). Denudation of endothelium reduced E_{max} of ADM from 59 ± 5% to 27 ± 6%. L-NAME, a nitric oxide (NO) synthase inhibitor or ODQ, a guanylate cyclase inhibitor, reduced the E_{max} of ADM from 60 ± 8% to 30 ± 8% (L-NAME), 21 ± 5% (ODQ), respectively. Moreover, K^+ -channel blocker, TEA, inhibited ADM–induced relaxation (E_{max} from 66 ± 8% to 17 ± 7%).

CONCLUSION: 1) Pregnancy and E_2 increases ADM –induced relaxation of UA, 2) pregnancy increases mRNA of $RAMP_3$ while E_2 increases both $RAMP_2$ (AM_1) and $RAMP_3$ (AM_2); 3) Mechanism of ADM–induced relaxation of UA is endothelium–dependent, NO-cGMP pathway –mediated, through opening of K_{Ca}–channels.

571

Local Uteroplacental Influences during Pregnancy Are Requisite for Vascular Structural Remodeling in the Uterus of the Rat. Robert R Fuller, Natalia I Gokina, George Osol.* *Department of Obstetrics and Gynecology, University of Vermont College of Medicine, Burlington, VT, USA.*

Objective. Vascular remodeling during pregnancy involves profound structural and functional modifications to the uterine circulation. While the mechanisms responsible for these changes remain unknown, this remodeling facilitates the dramatic increase in uterine blood flow that is essential to normal pregnancy outcome. The objective of this study was to use a single gravid-horn model to differentiate between the local and systemic influences of placentation and fetal development on uterine vascular structural remodeling during pregnancy in the rat.

Study design. Sprague-Dawley rats (N=34) underwent unilateral uterine horn ligation to create animals with single uterine horn pregnancies. Uteri were harvested on day 20 of gestation. The vascular morphology of the mesometrial area of the uterus (pregnant and non-pregnant horns) was evaluated, accompanied by specific measurements of the main uterine artery (e.g., length, diameter). In view of the known adaptive changes that occur in the smaller uterine resistance arteries during pregnancy, arcuate arteries were further evaluated by isolated-vessel distensibility studies using standard videomicroscopy techniques.

Results. Significant differences in structural remodeling were observed between the pregnant and non-pregnant uterine horns. The mesometrial arcade area was 7.8-times greater (1790 ± 290 vs 230 ± 73 mm^2, $p<0.001$) in the pregnant horn, uterine artery length was 2.5-times greater (103 ± 9 vs 41 ± 7 mm, $p<0.001$), and its non-pressurized inner diameter was 43% larger (219 ± 44 vs 153 ± 34 um, $p<0.001$). In addition to increases in caliber, arcuate arteries in the pregnant horn also demonstrated a 3- to 4-fold increase in distensibility coefficients ($p<0.001$) and a 15% increase in overall distensibility ($p<0.01$). Values are mean ± SD.

Conclusions. In summary, the single-horn pregnancy model is useful for differentiating local vs systemic influences on vascular remodeling in the pregnant uterus. Our data clearly demonstrate that local influences play the dominant role in expansive uterine vascular remodeling that is essential for normal outcome. These local influences affect both the cellular (mitotic) and the acellular (matrix) components of the remodeled vasculature.

572

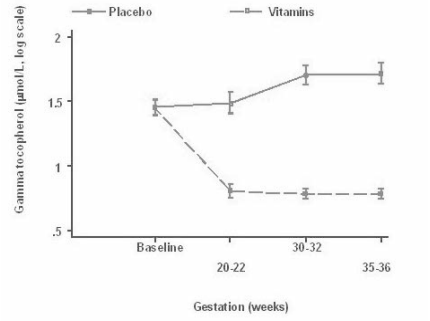
Why Do Vitamins C and E Not Prevent Pre-Eclampsia? Investigations into the Role of Oxidative Stress, Endothelial and Placental Dysfunction in the Disease. Lucy C Chappell,[1] Frank J Kelly,[2] Richard J Naftalin,[3] Beverley J Hunt,[4] Annette L Briley,[1] Paul T Seed,[1] Andrew H Shennan,[1] Lucilla Poston.*[1] *[1]Maternal and Fetal Research Unit, King's College London School of Medicine, London, United Kingdom; [2]Pharmaceutical Science Research Division, KCL, London, United Kingdom; [3]Cardiovascular Division, KCL, London, United Kingdom; [4]Department of Haematology, Guy's King's and St Thomas' School of Medicine, London, United Kingdom.*

Objective: Recent trials (Poston et al 2006, Rumbold et al 2006) showing that vitamins C and E do not prevent pre-eclampsia may challenge a role for oxidative stress in this disorder. We have now investigated plasma indices of oxidative stress, endothelial and placental dysfunction from women who took part in the UK Vitamins in Pre-eclampsia Trial.

Methods: Blood samples were taken in a subgroup of 192 women at randomisation and 20, 30 and 36 weeks' gestation, 41 of whom developed pre-eclampsia. Analytes were measured by standard laboratory techniques.

Results: Despite higher plasma triglyceride levels in those with subsequent pre-eclampsia, there was no difference in malondialdehyde concentrations and oxidised LDL antibodies were decreased ($p=0.042$ vs. normal outcome). However, plasma ascorbate concentrations in women with subsequent pre-eclampsia were lower {$p<0.05$), whilst plasma uric acid concentrations were increased, as was agonist stimulated superoxide production from isolated neutrophils (both $p<0.05$) and glucose-dependent mercaptoethanol production ($p<0·001$), an indicator of glutathione turnover. Supplemented women demonstrated a significant ($p< 0.001$) decrease in plasma γ-tocopherol (Figure 1), a potent antioxidant with anti-inflammatory properties. No difference was observed in plasminogen activator inhibitor (PAI)-1/PAI-2 ratio, considered an indicator of endothelial and placental dysfunction.

Conclusion: This study shows equivocal evidence for overt oxidative stress in the maternal circulation prior to clinical manifestations of the disease. The striking decrease in γ-tocopherol in supplemented women raises important questions about the antioxidant regime used, relevant to all trials using α-tocopherol.



573

The Inflammatory Cytokines Induce Interleukin-11 Expression in Human Decidual Cells; Implications for Preeclampsia. Chih-Feng F Yen,[1,2,3] Murat Basar,[1] Lynn Buchwalder,[1] Hakan Cakmak,[1] Umit A Kayisli,[1] Aydin Arici,[*1] Frederick Schatz,[*1] Charles J Lockwood.[*1] *[1]Ob, Gyn & Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA; [2]Ob & Gyn, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, Taiwan; [3]Graduate Institute of Clinical Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan, Taiwan.*

Objective: Preeclampsia (PE) is associated with an aberrant maternal immune response with restricted trophoblast invasion of first trimester decidua that leads to impaired spiral artery remodeling. Interleukin-11 (IL-11) mediates decidualization, placentation and inflammation. The pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and thrombin are involved in the pathogenesis of PE. We hypothesized that these cytokines may induce IL-11 expression in first trimester decidual cells (FTDC).

Methods: Immunohistochemistry and HSCORE were used to evaluateIL-11 levels in preeclamptic (n=4) versus normal decidua (n=4). Cultured FTDCs (n=8) were treated with estradiol (E2) or E2 + medroxyprogesterone acetate (MPA), to mimic the gestational hormonal milieu, +/- IL-1β, TNF-α, or thrombin. IL-11 levels in conditioned media were measured by ELISA. IL-11 mRNA levels were determined by quantitative RT-PCR (qRT-PCR). Statistical significance was examined using Student's t-test and ANOVA followed by post hoc test.

Results: IL-11 was markedly higher in decidual cells of preeclamptic versus normal specimens (HSCORE: 152.5±11.6 and 78.3±19.2, respectively; $P<0.05$). In comparison to FTDC cultured with E2 as a baseline, IL-11 secretion was induced by IL-1β and thrombin (168.5 ± 57.4- and 21.9 ± 5.3–fold, respectively; $P < 0.05$), whereas the effect was blunted by MPA (68.7 ± 22.3-fold and 12.3 ± 2.8-fold, respectively; $P < 0.05$). MPA totally inhibited the TNF-α-induced IL-11 secretion (16-fold before adding MPA vs. 1-fold after, of baseline). Corresponding effects on IL-11 mRNA levels were demonstrated by qRT-PCR.

Conclusions: IL-11 induced by pro-inflammatory cytokines in FTDC may play a crucial role in the pathogenesis of PE. Since a progestin was shown to protect against preterm delivery in a clinical trial, inhibition of cytokine-enhanced IL-11 expression in FTDC by MPA may mimic such protective effects.

574

Microparticles (MPs) Shed from Human Placenta Contain Anti-Fibinolytic and Anti-Angiogenic Compounds: Implications in Preeclampsia. Seth Guller,[*1] Yuehong Ma,[1] Stefano Di Santo,[2] Graciela Krikun,[*1] Antoine Malek,[2] Henning Schneider.[*2] *[1]OB/GYN, Yale University School of Medicine, New Haven, CT, USA; [2]OB/GYN, Universitäts-Frauenklinik, Inselspital, Berne, Switzerland.*

INTRODUCTION: Microparticles (MPs) shed from placenta to maternal blood are implicated in the pathophysiology of preeclampsia (PE). The protein composition of MPs remains largely unelucidated. Placental production of the anti-fibrinolytic factor plasminogen activator inhibitor-1 (PAI-1), and the anti-angiogenic factor soluble fms-like tyrosine kinase (sFlt-1), are suggested to dysregulate hemostasis and angiogenesis in PE. The function of PAI-2, the "placental PAI", remains unelucidated. Our objective was to determine whether placental MPs released during dual (maternal and fetal) perfusion and by cultures of syncytiotrophoblasts (SCTs) contain PAIs and sFlt-1.

METHODS: Dual perfusion of human term placentas (n=5) was carried out for 7 h. Perfusate was recirculated on both maternal and fetal sides, the media was replaced at 1 and 3 h, and samples of maternal perfusate were taken at 1, 3 and 7 h. Conditioned media were obtained from SCTs cultured for 48 h (n=3). MPs were collected from maternal perfusate and conditioned media by centrifugation, and proteins were solubilized by treatment with Triton X-100 detergent. Levels of PAI-1, PAI-2, and sFlt-1 were evaluated by ELISA.

RESULTS: Between 1 and 7h of perfusion the level of PAI-1 in MPs from maternal perfusate increased from 1.2±0.6 to10.2.±3.8 pg/g tissue/min ($P<0.05$). PAI-2 levels in MPs were 557±255 and 933±385 at 1 and 7h, respectively. PAI-1 and PAI-2 levels in the soluble fraction of maternal perfusate were equal, suggesting "preferential packaging" of PAI-2 in MPs. sFlt-1 levels in MPs were 2.4±0.8 and 1.3±.0.4 at 1 and 7h of perfusion. The level of PAI-1, PAI-2, and sFlt-1 in MPs from cultures of SCTs were 26.6.± 3.8, 27.8±1.7, and 2.0±0.1 pg/µg protein, respectively.

CONCLUSIONS: 1) MPs in maternal perfusate contain PAI-1, PAI-2, and sFlt-1. 2) MP PAI-2 levels were 50-fold higher than PAI-1 and sFlt-1, indicating preferential sequestration. 3) PAI-1 MP levels increased during perfusion, reflecting placental stress previously noted by our group. 4) The presence of these proteins in MPs from cultured SCTs indicates a syncytial origin during perfusion. This study suggests that MPs are a source of proteins that promote aberrant patterns of fibrinolysis and angiogenesis in PE.

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575

Effect of Hypoxia on Phosphorylation of cAMP Response Element-Binding (CREB) Protein in the Neuronal Nuclei of the Guinea Pig Fetus during Gestation. Dev Maulik,[*1] Qazi M Ashraf,[2] Om P Mishra,[2] Maria Delivoria-Papadopoulos.[*2] *[1]Obstetrics and Gynecology, Winthrop University Hospital, Mineola, NY, USA; [2]Pediatrics, Drexel University College of Medicine, Philadelphia, PA, USA.*

Previous studies in neuronal cells have shown that CREB protein is a target for calcium /calmodulin (CaM) dependent protein kinase, leading to phosphorylation of CREB on its activator site serine[133], a marker for CREB-mediated transcription. We have also shown that hypoxia results in increased phosphorylation of CREB protein at Ser^{133} in the cortical neuronal nuclei mediating gene expression including that of the pro-apoptotic bax.

The present study tests the hypothesis that inhibition of NOS by N-nitro-L-arginine methyl ester (L-NAME) will prevent the hypoxia-induced increased phosphorylation of CREB (p-CREB) protein in neuronal nuclei of the guinea pig fetus during gestation.

32 guinea pig fetuses at 35 and 60 days gestation were assigned to normoxic (Nx, n=6), hypoxic (Hx, n=6) and hypoxic pretreated with nitric oxide synthase inhibitor (Hx+L-NAME, 30mg/kg i.p., n=4) groups. Hypoxia in the fetuses was induced by exposing the pregnant guinea pigs to a FiO_2 of 0.07 for 60 min. Fetal cortical nuclei were isolated. Hypoxia was documented by levels of ATP and Phosphocreatine. Nuclear proteins were immunoblotted with anti-p-CREB antibody. Density ($ODxmm^2$) of Ser^{133} p-CREB protein in 35d was 61.6±2.2 in the Nx compared to 98.8±3.7 in Hx ($p<0.05$) and 75.7±2.7 in the Hx-L-NAME group. Density of Ser^{133} p-CREB protein in 60d was 107.7±9.2 in the Nx compared to 273.3±9.4 in the Hx ($p<0.05$) and 171.2±11.9 in the Hx-L-NAME group. The data show that NOS inhibition attenuates the hypoxia-induced increase in CREB protein phosphorylation in cortical nuclei of preterm and term fetuses. CREB phosphorylation was higher in term gestation compared to preterm. We conclude that the hypoxia-induced increase in CREB phosphorylation is NO-mediated. Increased phosphorylation of CREB protein at term indicates a high rate of ongoing programmed cell death in the fetal brain due to synaptic innervation and pruning at term as compared to preterm.

We propose that NO-mediated increase in intranuclear Ca^{2+} during hypoxia leads to increased activation of CaM kinase IV resulting in increased phosphorylation of CREB that triggers transcription of the apoptotic gene Bax. (NIH-HD 20337, NIH-HD 38079 and St. Christopher's Foundation for Children).

576

Altered Expression of Regulators of Caspase Activity within Trophoblast of Normal and Pre-Eclamptic Pregnancies. Alexander E Heazell, Hilary R Buttle, Ian P Crocker, Philip N Baker.[*] *Divison of Human Development, University of Manchester, Manchester, United Kingdom.*

Background: Apoptosis within the villous trophoblast is increased within pregnancies complicated by pre-eclampsia (PE), this is associated with an increase in the expression and activity of caspases 3 and 8. Caspase activity is negatively regulated by inhibitors of apoptosis proteins (IAPs). The intrinsic

apoptotic pathway is activated following cell damage, resulting in increased mitochondrial permeability, releasing factors into the cytosol. Two of these, smac and omi, inhibit the action of IAPs, increasing caspase activity. We hypothesized that the increased apoptosis and caspase activity in PE would be associated with an imbalance between smac / omi and IAPs.

Objectives: This study was designed to assess the expression and localisation of smac, omi and IAPs (XIAP and survivin) in placentae of normal pregnancy and PE.

Methods: 5 random placental biopsies were taken immediately after delivery of 8 normal pregnancies and 8 with PE. The tissue was homogenised, and Western Blotting performed on the lysate. The presence of smac, omi, XIAP or survivin was assessed using densitometry compared to myosin light chain and β-actin. Immunohistochemistry was undertaken on fixed samples.

Results: In normal pregnancies, smac immunostaining was evident in syncytiotrophoblast (ST), cytotrophoblast (CT) and endothelial cell cytoplasm. Smac was significantly elevated in PE compared to normal pregnancies (Ratio median 0.72, Interquartile Range (IQR) 0.52-0.83 vs 0.48, 0.29-0.66 $p<0.05$) this was associated with increased staining in ST cytoplasm. We could not detect omi using western blotting, although weak immunostaining was present in ST cytoplasm. There was no alteration in XIAP expression (Median 0.20, IQR 0.17-0.34 vs 0.31, 0.10-0.45, $p>0.05$). XIAP localized to ST cytoplasm, with weak immunostaining in CT cytoplasm. The expression of survivin was not altered in PE. Unlike XIAP, survivin not only localized to ST and CT cytoplasm, but also nuclei of mitotic CT.

Conclusions: There is an alteration in the balance of smac and IAPs in the the ST of pregnancies complicated by PE. Increased expression of smac in the presence of normal levels of XIAP and survivin can induce apoptosis and increase caspase activity. Therefore, the observed imbalance may be important in the increased placental caspase activity and apoptosis observed in PE.

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577

Differential Gene Expression Profiling in HELLP Syndrome Placentas. Bum-Yong Kang, Shan Zhu, Shenghui Su, Helen H Kay,* Stephen CM Tsoi.* *Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.*

Objective: HELLP syndrome is a unique variant of severe preeclampsia characterized by hemolysis, elevated liver enzymes, and low platelet count. Identification of differential gene expression profiling in HELLP placentas may be a critical step leading to the elucidation of molecular mechanisms and biomarkers development of HELLP syndrome. Transcriptome analysis of HELLP placentas still remains uninvestigated. This study aimed to identify differentially expressed genes in HELLP placentas.

Methods: Total RNA was extracted from HELLP (n=2) and non-preeclampsia placentas (n=2). cDNAs were synthesized from pooled mRNAs (1.0 μg from each sample) using suppression subtractive hybridization (SSH). 288 positive clones from each subtracted cDNA library were selected for single pass sequencing. The differential expression of selected genes was further validated by either semi quantitative RT PCR or real-time quantitative PCR.

Results: Forty-three were unique clones. Among them, nine HELLP syndrome **induced** genes were pregnancy specific beta-1-glycoprotein 4, PSG4; heat shock protein 90kDa beta, HSP90B1; zinc finger protein 326, ZNF326; tissue factor pathway inhibitor 2, TFPI2; glycoprotein hormones alpha polypeptide, CGA; eukaryotic translation elongation factor 1 alpha 1, EEF1A1; PHD finger protein 17,PHF17; annexin A1, ANXA1; and SAM and SH3 domain containing 1, SASH1; and eight HELLP syndrome **suppressed** genes were serpin peptidase inhibitor, SERPINE2; single-stranded DNA binding protein 1, SSBP1; keratin 8, KRT8; keratin 6A, KRT6A; adipose differentiation-related protein, ADFP; adiponectin receptor 1, ADIPOR1; Kruppel-like factor 4, KLF4; and DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked, DDX3X.

Conclusions: This study represents the first systematic molecular profiling of HELLP syndrome placentas. ANXA1, HSP90B1and PHF17 have been related to apoptosis in cancer. These can be candidate genes for further understanding the apoptotic pathway related to HELLP syndrome. The soluble protein products related to PSG4, TFPI2, SERPINE2, EGFL6 can develop into blood biomarkers to detect the onset of HELLP syndrome. Therefore, further investigation of these differentially regulated genes from HELLP placentas could shed light on the etiology and pathogenesis of HELLP syndrome and help develop genetic markers for early diagnosis and possibly therapeutic targets.

578

Differential Cerebral Blood Flow and Blood-Brain Barrier Permeability during Acute Hypertension in Pregnancy and Its Role in Eclampsia. Marilyn J Cipolla,* Lisa Vitullo, Anna G Euser. *Neurology, Ob/Gyn and Pharmacology, University of Vermont, Burlington, VT, USA.*

Objective: Eclampsia is thought to be similar to hypertensive encephalopathy in which an acute elevation in blood pressure causes autoregulatory breakthrough, hyperperfusion and brain edema. We previously reported that while the pressure of breakthrough was similar between nonpregnant and late-pregnant (LP) rats, only LP animals developed edema. In the present study, we investigated how acute hypertension affects blood-brain barrier (BBB) permeability and cerebral blood flow (CBF) in LP rats, primary mechanisms by which brain edema forms.

Methods: In vivo models of BBB permeability and CBF were used in LP (d19-20) rats that were either normotensive (111±1 mmHg; n=8) or hypertensive by infusion of phenylephrine to raise mean arterial pressure (163±2 mmHg; n=8). Permeability was determined by clearance of Evan's blue dye (2%) into the brain tissue, measured by a fluorescent spectrophotometer after flushing the vasculature with saline and homogenization of the brain tissue. CBF was similarly measured by infusion of 15μm fluorescent microspheres and calculated based on the flow rate and fluorescence intensity of a reference sample for each animal. BBB permeability and CBF were determined in the anterior and posterior regions of the brain. Animals were ventilated to control blood gases within normal (PO_2>100mmHg, PCO_2 =35-45mmHg).

Results: Acute hypertension increased CBF in both the anterior and posterior regions of the brain from 63±6 to 292±39 mL/min/100g tissue ($p<0.01$) and 65±6 to 277±35 mL/min/100g tissue ($p<0.01$), respectively. While hypertension increased BBB permeability in both regions, the increase in permeability was considerably greater in the posterior region. Permeability increased 308% in the anterior region from 341±221 to 1390±323 counts/g tissue ($p<0.05$) and 590% in the posterior region from 390±133 to 2687±453 counts/g tissue ($p<0.05$).

Conclusions: These data demonstrate that acute hypertension causes significant hyperperfusion of the brain during pregnancy that is similar between the anterior and posterior regions. However, permeability was significantly greater in the posterior region, suggesting that changes in permeability are not related to changes in CBF. The increased permeability in the posterior region also suggests differential tight junction expression in the BBB that may relate to the propensity for edema to form in that area during eclampsia.

579

The Mechanism of the Developmental Origin of Adult Hypertension in an Animal Model of Preeclampsia. Fangxian Lu, Egle Bytautiene, Esther H Tamayo, Garland D Anderson, Gary Hankins,* Monica Longo, George R Saade. *Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, TX, USA.*

Objective: We have previously validated a model of sFlt-1-induced preeclampsia in mice, and shown that the adult offspring born to these mice are hypertensive. Our objective with this study was to further investigate the mechanisms involved in this fetal programming of blood pressure by evaluating vascular function in the adult offspring.

Method: As previously established, CD-1 mothers at day 8 of gestation were injected with an adenovirus carrying Flt (1-3) [AdFlt(1-3); 10^9 PFU] or with an adenovirus carrying mFc as control (10^9 PFU). The resulting female and male offspring were followed until 3 months of age (average life span 1.5 years) at which time they were sacrificed (n=5/group), and 2 mm segments of carotid artery were mounted in a wire myograph for isometric tension recording. Responses to KCL were determined and concentration-response curves to acetylcholine (Ach, 10^{-10}-10^{-5} M), sodium nitroprusside (SNP, 10^{-10}-10^{-5} M), phenylephrine (PE, 10^{-10}-10^{-5} M), and thromboxane A2 (TxA2, 10^{-12}-10^{-6} M) were obtained. One-way ANOVA followed by Newman-Keuls post-hoc test were used for statistical analysis (significance: $p<0.05$).

Results: At 3 months of age, both female and male offspring born to sFlt-1-treated mother had significantly higher response to KCL compared with the offspring born to their own control groups (4.3±0.23 mN and 3.74±0.25 mN vs 3.47±0.24 mN and 2.99±0.28 mN, $p<0.05$). There were no differences in KCl responses between genders within each treatment group. In addition, no significant differences in vascular responses to any of the other agents used (PE, TXA2, Ach and SNP) were noted in both male and female offspring.

Conclusion: Given that the effect of sFlt-1 over-expression was limited to the KCl responses, it is likely that the underlying mechanism of fetal programming in our animal model of preeclampsia involves structural rather than functional pathways.

580

IL8 Promotes Trophoblast Cell Invasion *In Vitro*. Leandro G Oliveira,[1] Gendie E Lash,[1] Harry A Otun,[1] Barbara A Innes,[1] Katsuhiko Naruse,[1] Roger F Searle,[2] Stephen C Robson,[*1] Judith N Bulmer.[3] *[1]SARS, Newcastle University, Newcastle, United Kingdom; [2]MED, Newcastle University, Newcastle, United Kingdom; [3]CALS, Newcastle University, Newcastle, United Kingdom.*

Background: During early pregnancy extravillous trophoblast cells (EVT) invade decidua as far as the inner third of myometrium. This is a tightly regulated process involving decidua derived cytokines and chemokines. Recent studies have suggested a role for IL8 in this process; the source within decidua and mechanisms of action are largely unknown.

Hypotheses: 1) IL8 is secreted by decidual cells in early human pregnancy. 2) IL8 stimulates EVT invasion *in vitro* via a mechanism dependent on increased protease activity.

Methods: CD8+ T lymphocytes, total decidual and uterine natural killer (uNK) cells prepared by enzymatic disaggregation were positively selected using an immunomagnetic technique (8-10 and 12-14 weeks gestation; n=5 both groups). CD8+ cells were incubated with phytohaemagglutinin (PHA-P; 10mg/ml) for 24h. Total decidual and uNK cells were cultured for 24h in the absence of PHA-P. Supernatants were harvested and IL8 protein levels determined by ELISA. Immunohistochemistry for IL8 receptors (CXCR1 and CXCR2) was performed on first trimester placental bed biopsies. Matrigel invasion assays were performed with placental explants (8-10 weeks) for 6 days under the following conditions: medium alone, IL8 (0.1, 1 and 10 mg/ml), IL8 neutralizing antibody (nAB) and IL8 10 mg/ml + IL8 nAB. Each experiment was performed in triplicate (n=7). At the end of the invasion assay the medium was harvested and levels of secreted MMP-2 and MMP9 determined by gelatin zymography.

Results: High levels of IL8 protein were detected in total decidual cell (8-10w 117.7 ± 66.2 µg/ml; 12-14w 219.6 ± 120.6 µg/ml), uNK (8-10w 11.7 ± 3.3 µg/ml; 12-14w 40.6 ± 25.9 µg/ml) and decidual CD8+ T lymphocytes (8-10w 160.2 ± 72.1 µg/ml; 12-14w 195.3 ± 130.3 µg/ml) supernatants. CXCR1 and CXCR 2 were expressed by interstitial, endovascular and intramural EVT. IL8 increased EVT invasion (0.1 ng/mL (P<0.02) and 10 ng/mL (P<0.03)) compared to controls. No difference in levels of secreted MMP-2 and MMP-9 was observed under any of the experimental conditions.

Conclusion: CD8+ T Lymphocytes and uNK cells are both sources of IL-8 in early human pregnancy decidua and receptors for this chemokine are present on EVT cells. IL8 increases EVT invasion *in vitro* through mechanisms that do not involve altered MMP-2 and MMP-9 activity.

581

Trophoblast Stem Cells Induce IFNγ Production by LAK Cells in Part by NKG2D. Joan K Riley,[1] Leon N Carayannopoulos,[2] Jennifer L Barks,[2] Wayne M Yokoyama,[2] Kelle H Moley.[*1] *[1]Obstetrics and Gynecology; [2]Internal Medicine, Washington University, St. Louis, MO, USA.*

Uterine natural killer (uNK) cells are the predominant lymphocyte found in the gravid uteri. Studies conducted in mice have demonstrated that interferon gamma (IFNγ) is the critical molecule derived from uNK cells that is required for the remodeling of uterine spiral arteries by trophoblast cells and for optimal decidualization. Objective: Investigate the mechanism(s) underlying uNK cell activation leading to the production of trophic factors required for successful implantation. Methods/Results: We established an in vitro system to model trophoblast-NK cell interactions. Trophoblast stem (TS) cells were derived from murine C57BL/6 blastocysts. We found by flow cytometry or confocal microscopy that both TS cells and blastocysts express RAE-1. RAE-1 is a ligand for NKG2D, an activating receptor expressed on NK cells. In mice there are three NKG2D ligands RAE-1, MULT1 and H60 of these only RAE-1 is expressed on the blastocysts and TS cells. NKG2D activation by its ligands is known to lead to IFNγ production. We cocultured splenic NK cells that were propagated in IL-2 (LAK cells) with TS cells and found that the TS cells induced IFNγ production (maximal response=350 pg/ml) by LAK cells as determined by ELISA. All experiments described herein were performed three times. We also differentiated TS cells into trophoblast giant cells and found they also induced IFNγ production by LAK cells. DAP10 and DAP12 are adapter molecules that transduce signals via NKG2D and other NK cell activating receptors. IFNγ production by LAK cells derived from DAP10xDAP12 double knockout mice was reduced by approximately 50% (150 pg/ml) when cultured with TS cells as compared to controls. Thus IFNγ produced in this system occurs by DAP10/DAP12 dependent and independent mechanisms. To determine whether the DAP10/DAP12 dependent pathway of IFNγ production was due to the interaction of NKG2D with RAE-1 we performed a ligand blocking experiment. LAK cells were preincubated with soluble MULT1 and then cocultured with TS cells. Preincubation of the LAK cells with MULT1 lead to a complete block in the DAP10/12 dependent mode of IFNγ production. Conclusion: Activation of NKG2D by RAE-1 is responsible for the DAP10/DAP12 dependent mechanism of IFNγ production in this TS-NK cell coculture system. Studies to determine the in vivo relevance of these findings with respect to IFNγ production by uNK cells are ongoing.

582

Effects of Low Molecular Weight Heparin on Trophoblast Invasiveness. Nicoletta Di Simone, Maria Clara D'Alessio, Fiorella Di Nicuolo, Roberta Castellani, Silvia D'Ippolito, Alessandro Caruso. (SPON: Irene Cetin). *Obstetrics and Gynecology, Catholic University of Sacred Heart, Rome, Italy.*

Objective: The purpose of this study was to investigate the in vitro ability of low molecular weight heparin (LMWH) to affect trophoblast invasiveness. Although the mechanisms of placental invasion are not elucidated, it is reported that matrix metalloproteinases (MMPs) and the tissues inhibitors of metalloproteinases (TIMPs) are related to this invasion. Moreover, heparin-binding-EGF (HB-EGF) has been found to be important in early implantation and deficient HB-EGF signalling during placental development could impair trophoblast functions. Therefore, we investigated if LMWH might regulate trophoblast invasiveness, placental production of MMPs, TIMPs, and HB-EGF expression. Since there is evidence that antiphospholipid antibodies (aPL) may directly inhibit the trophoblast invasion, we investigated if IgG aPL might affect placental expression of HB-EGF, and then if LMWH might be able to restore HB-EGF expression. **Methods:** We used trophoblast cells obtained from spontaneous abortions and choriocarcinoma cells. Cell cultures were performed for 24 hrs in medium containing LMWH (0.1-10 IU/ml) and/or IgG anti-b2-glycoprotein I (25-100 µg/ml). The invasive potential of trophoblast cells was examined using a membrane invasion culture system. The determination of MMP-2 and TIMP-1 and -2 in cell culture supernatants after treatment with LMWH was performed by ELISA. Expression of TIMPs, MMP-2, and HB-EGF was studied by Western blot analysis and by Real-Time PCR. **Results:** LMWH significantly increased trophoblast cells invasiveness and enhanced both pro-MMP-2 and its active form. LMWH effects were also indirect, inducing a significant decrease in TIMPs protein expressions. Treatment of trophoblast cells with LMWH, resulted in stimulation of HB-EGF as protein and mRNA levels. Finally we found that anti-b2-glycoprotein I reduced the placental expression of HB-EGF, and heparin restored the protein expression. **Conclusions:** Our results indicate that heparin is able to regulate several proteins involved in trophoblast invasiveness. The increase in HB-EGF might be able to induce the stimulation of MMPs, which promote trophoblast invasiveness. We would like to suggest that the decline in TIMPs expression, induced by LMWH, could remove the inhibitory influence on MMPs activity. These data suggest a role for LMWH on trophoblast proteins playing a central role in placental invasion.

583

Proteomic and Biochemical Evidence Indicates a Role for Tissue Transglutaminase (tTG) in Particle Shedding from the Placenta. Nicola J Robinson, Philip N Baker,[*] Carolyn JP Jones, John D Aplin. *Maternal and Fetal Health Research Centre, The University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Objectives: Despite its widespread expression, the precise biological role of the bifunctional cross-linking enzyme tissue transglutaminase (tTG) is unclear. We have previously demonstrated strong reactivity at the syncytial microvillous membrane (MVM). Using a proteomic approach, here we demonstrate a role for tTG in trophoblast in the processing of membrane and cytoskeletal material destined to be shed from the placenta prior to clearance by the maternal immune system.

Methods: In order to identify tTG substrates in the MVM, membrane vesicles were prepared and labelled with biotinylated acyl donor or acceptor probes. Biotinylated species were selected on an avidin affinity matrix and identified by LC-MSMS of tryptic peptides. To test the hypothesis that material shed from terminally differentiated syncytiotrophoblast may be cross-linked by tTG, primary cytotrophoblasts were cultured, and the material shed from the monolayer cultures between 18-66h was isolated by differential centrifugation. The competitive substrate inhibitor monodansylcadaverine or cystamine, an active site inhibitor of tTG were added to the cultures to examine the role of tTG in shedding.

Results: The most abundant tTG substrates identified in the MVM were cytoskeletal (actin, tubulin, cytokeratin) and membrane-associated (annexins, integrins, placental alkaline phosphatase, transferrin receptor) proteins. Syncytial microparticles shed from the trophoblast cultures were found to contain abundant tTG protein and cross-linking activity. A substantial fraction of actin in the shed particles was in the form of covalent polymeric aggregates, in contrast to cellular actin, which dissociated completely into monomer in SDS-PAGE. When cells were cultured in the presence of transglutaminase inhibitors, actin in particulate material shed into the culture medium remained exclusively in monomeric form.
Conclusions: These results suggest that transglutaminase-mediated cross-linking may stabilize the particulate material shed from the placenta, a process that likely contributes to the low maternal immune reaction to high quantities of fetal antigen.

584

Systemic Maternal Awareness of Conceptus Antigens in Pregnancy. Sarah A Robertson,[1] Lachie M Moldenhauer,[1] John D Hayball.[2] (SPON: Joan S Hunt). *[1]Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia; [2]Samson Institute, University of South Australia, Adelaide, SA, Australia.*
Objective: The extent to which conceptus immune rejection in pregnancy is avoided by failure to activate immune recognition, as opposed to protection by active immune tolerance, is not clear. This study utilized T-cell transgenic mouse models with ovalbumin (OVA) as a model paternal antigen to investigate the occurrence, kinetics and location of paternal antigen priming in pregnancy, and to evaluate whether presence of paternal antigen-reactive cytotoxic T-cells is consistent with viable pregnancy.
Methods: Act-mOVA male mice constitutively expressing OVA were mated to B6 females, to generate OVA+ conceptuses in OVA-deficient mothers. Pregnant mice received CFSE-labelled OVA-reactive OT-1 T cells on gestation day (gd) 1, 4, 7, 11 or 15. OT-1 cell proliferation and CD69 expression were quantified to gauge OVA antigen processing and presentation at 3 days after transfer. Additional B6 females mated with Act-mOVA males were given CD8+ OT-1 cells on gd 1 and pregnancy outcome was assessed at gd 18.
Results: OT-1 cells given on gd 1 showed marked activation and proliferation in the para-aortic lymph nodes draining the uterus. OT-1 cells given on gd 4 displayed very little activation or proliferation in any lymphoid tissues. From gd 7, OT-1 cell activation and proliferation increased in the para-aortic nodes, and over the course of pregnancy from gd 11 onwards, progressively spread to peripheral sites including the mesenteric and cervical nodes and the spleen. Administration of OT-1 cells on gd 1 did not adversely affect litter size, resorption rates or fetal and placental weights on gd 18.
Conclusion: Paternal antigens associated with both semen and the conceptus actively prime the maternal immune system, with the response becoming strong and systemic by mid-late gestation. This provides a mechanism whereby semen provides the initial priming event for paternal antigen recognition, and the placenta sustains this response after implantation, potentially maximising systemic antigen deposition by shedding trophoblast microparticles. Failure of paternal antigen-reactive cytotoxic T-cells to adversely affect pregnancy is consistent with a role for active maternal tolerance in preventing fetal rejection. Exploiting this model will allow investigation of the events underpinning establishment and maintenance of the maternal immune tolerance facilitating pregnancy success.

585

CD8 Anti H-Y T Cells in Pregnancy. Michelle Norton, Manjula Santhanakrishnan, Peyman Bizargity, Elizabeth A Bonney.* *OB/GYN, University of Vermont College of Medicine, Burington, VT, USA.*
Background: Our understanding of pregnancy's influence on maternal cell-mediated immunity is incomplete. While pregnant, mice can reject male skin grafts, yet do not abort their male pups. To determine underlying mechanisms, we have begun to study transgenic mice whose CD8 T cells are exclusively specific for the male antigen H-Y.
Objective: To observe the level of CD8 T cells in the spleen of pregnant H-Y specific T cell receptor (TCR) transgenic mice.
Methods: Female C57BL/6, *rag*-1$^{-/-}$, CD8+ Vβ8.3+ anti-H-Y TCR transgenic mice (Matahari) were used unmated (UM) or underwent mating with same-strain males and euthanized 8- 18 days post coitus. Harvested spleen was used to prepare single cell suspensions that were enumerated, incubated with antibodies to CD8, Vβ8.3 TCR, and CD44 and analyzed by flow cytometry to determine the percentage and number of CD8+ Vβ8.3+ cells and their expression of CD44. The averages obtained for groups of mice from each gestational day were compared to that in UM mice by ANOVA, with significance set at $p<0.05$.
Results: Matahari mice, as compared to non-transgenic mice (NT), have similar litter sizes (Matahari, 5.8±1.6 pups, n=14 vs. NT, 5.3±2.7, n=27 p=0.47), and deliver similar proportions of male pups (Matahari, 0.49±0.22 vs. NT, 0.47±0.28, p=0.78). When we assessed the numbers of CD8+ Vβ8.3+ cells in Matahari spleen during pregnancy, we found that on Day 8 there was a significant increase compared to UM females ($6.98\pm2.92x10^5$ n=3 vs. $3.59\pm1.58x10^5$ n=9 p= 0.024). This was followed by a significant decrease in these cells on Day 10 ($0.77\pm0.79 x10^5$ n=4 p= 0.0067). By day 14 the numbers had increased to UM levels ($4.34\pm0.56x10^5$ n=6 p=0.29). On day 18 the numbers again greatly increased ($10.34\pm3.51x10^5$ n=4 p=0.0004). Multiparous mice also had more CD8+ Vβ8.3+ cells than UM females ($9.74\pm5.48x10^5$ cells n=3 p=0.0037).
The proportion of CD8+ Vβ8.3+ cells that were positive for the activation and memory marker CD44 did not decrease in pregnancy. However, in multiparous mice the percentage of CD8+ Vβ8.3+ cells that were also CD44+ was significantly higher than in UM mice, (84.5±15.1 vs. 61± 9.9 p=0.01).
Conclusions: In this model pregnancy neither causes permanent loss of anti H-Y CD8 T cells, nor inhibits their expression of an activation antigen. Multiple pregnancies enhance both their numbers and activation. This argues against pregnancy as a state of immune limitation or paralysis. *Supported by NIHRO1HD043-185.*

586

Coordinated Up-Regulation of Pro-Inflammatory Pathways by Thrombin in Human Endometrial Endothelial Cells. Graciela Krikun,* ST Joseph Huang, Charles J Lockwood.* *Ob/Gyn & Rep. Sciences, Yale University, School of Medicine, New Haven, CT, USA.*
Background: Coagulation factors like thrombin, are important regulators of inflammation, aberrant vascular development, and angiogenesis in various systems including the female reproductive system. However, the molecular mechanisms by which these factors exert multiple pathologic effects remain unclear. The endothelial layer represents a physical barrier that controls coagulation and allows selective passage of soluble molecules and circulating cells across the vessel wall into the tissue. These functions are influenced by the surrounding environment. The current study utilized microchip gene arrays coupled to a network analysis program to discern the interaction between molecules upregulated by thrombin in human endometrial endothelial cells. The results were confirmed by quantitative RT-PCR as well as by ELISAs for several endpoints.
Methods: Cultured human endometrial endothelial cells were treated +/- 2.5 U thrombin and analyzed by Affymatrix microchip arrays, quantitative RT-PCR and ELISAs. Interrelated network analysis was carried out with the Ingenuity Pathways Analysis Program (Redwood City, CA)
Results: Thrombin exposure of endometrial endothelial cells resulted in a 357 fold induction of **E-selectin** a 70 fold induction of **VCAM 1**, a 35 fold induction of **CSF2**, and a 19 fold induction of **ICAM 1**. In addition, thrombin upregulated the expression of CCL20 (**MIP3a**) by a 2 log-fold. Following computerized network merging, it was observed that the activation of these proteins interconnected pathways involving cell-cell interaction and inflammation and control by ubiquitin-mediated protein degradation. It is interesting therefore that ubiquitin D was also upregulated by 186 fold after thrombin exposure. All endpoints showed similar upregulation by quantitative RT-PCR or by protein analysis.
Conclusion: We posit that pathologic levels of thrombin activate endometrial endothelial cells resulting in inflammatory disease for both the pregnant and non pregnant endometrium. This occurs by the upregulation of E-selectin, VCAM, ICAM, CSF2, CCL20 and ubiquitin D. Computerized analysis of our data demonstrates a link of inflammatory and apoptotic signaling pathways that include blood leukocyte recruitment, adhesion, rolling, binding, migration, infiltration, extravasation, chemotaxis and protein degradation.

587

Acute Hepatitis E Infection in Pregnancy Is Associated with High Maternal and Perinatal Mortality Rates. Nazli Hossain,[1] Nargis Soomro,[2] Tahir Shamsi,[3] Michael Paidas.[1] (SPON: Charles J Lockwood). *[1]Obstetrics Gynecology Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Obstetrics & Gynecology Unit 2, DUHS, Karachi, Pakistan; [3]Hematology, BTIHS, Karachi, Pakistan; [4]Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: Hepatitis E is an emerging public health problem in several parts of the world. The published literature suggests a 15% -20% case fatality rate in pregnancy. In this study, we sought to evaluate the pregnancy outcome of patients with Hepatitis E infection in one institution with 1400 deliveries annually.

Materials & **Methods:**From January 2006- September 2006, patients suspected of having liver dysfunction in pregnancy underwent screening for Hepatitis E in addition to laboratory evaluation for preeclampsia syndromes, intrahepatic cholestasis, acute fatty liver of pregancy (AFLP), hepatitis B and hepatitis C. Liver and renal function testing, as well as coagulation profiles were obtained in all patients.

Results : Thirty five women were identified with liver dysfunction based upon transaminitis. Fourteen (40%) of the women presented in the second trimester, and 22(60%) presented in the third trimester. Eight (22%) women were registered for antenatal care, where as 27(77%) were unregistered. Out of 35 women, 11(31%) were primigravid, 8(22%) were in their second pregnancy and 17(48%%) were multigravid. Twenty two of the 35 (62%) women had isolated acute hepatitis E; 5(14%) had HELLP syndrome; 2 (5%)had cholestasis and 2(5%) had AFLP. In women with hepatitis E, the mean value of bilirubin and SGPT were 12 mg/dl and 675 u/l respectively. Coagulation profile of the group was abnormal 20(57%)women, and in 18 of 22 (82%) with hepatitis E. Fulminant hepatic failure was seen in 5 (14%)patients, all of whom had hepatitis E. Seven women (20%) underwent cesarean section; 26(74%) delivered vaginally, and 2 women remained undelivered in the postmortem state. There were 6 maternal deaths in the study population; 4 (67%) were due to hepatitis E, and one each from HELLP and AFLP. The overall perinatal mortality of the group was 40%. Hepatitis E was associated with a 36% (8/22) preterm delivery rate, and 27% (6/22)rate of fetal demise.

Conclusion: . In this series, Hepatitis E was the most common cause of liver dysfunction, etiology of fulminant hepatic failure and maternal death. Patients with liver dysfunction should be screened for hepatitis E.

588

Effects of Asoprisnil, a Selective Progesterone Receptor Modulator (SPRM), in Women with Treatment Resistant Heavy Menstrual Bleeding. Hilary Critchley,[*1] Alistair Williams,[1] Iain Cameron,[*2] Mary Ann Lumsden,[3] Kristof Chwalisz,[*4] Cornelia Holz,[5] Jens Wessel.[5] *[1]Centre for Reproductive Biology, University of Edinburgh, Edinburgh, United Kingdom; [2]Developmental Origins of Health and Disease Division, University of Southampton, Southampton, United Kingdom; [3]Dept of Obstetrics and Gynaecology, University of Glasgow, Glasgow, United Kingdom; [4]TAP Pharmaceutical Products Inc, Lake Forest, IL, USA; [5]Jenapharm GmbH & Co6. KG, Jena, and Schering AG, Berlin, Germany.*

Introduction: Asoprisnil, a novel SPRM, induces reversible amenorrhoea without consistent suppression of ovulation. The objective of the present study was to explore the efficacy of asoprisnil for the management of treatment-resistant heavy menstrual bleeding (HMB: menorrhagia) without organic cause.

Methodology: A multi-centre, double-blind, placebo-controlled, randomized, parallel-group study was undertaken. 26 women seeking surgery for HMB participated (full analysis set; FAS). Women were randomized to receive placebo (n=8); 5mg asoprisnil (n=5); 10mg asoprisnil (n=7) or 25mg asoprisnil (n=6) daily for between 35 and 56 days prior to hysterectomy. The primary outcome was individual relative change in uterine bleeding score by pictorial blood loss assessment chart (PBAC).Endometrial morphology and immunohistochemistry (IHC) of sex steroid receptor expression (ERα and PR), in full-thickness endometrial samples were evaluated.

Results: All subjects showed variable degrees of HMB in the pre-treatment cycle. Within a maximal treatment period of 56 days asoprisinil induced a marked reduction in mean PBAC score compared with placebo [mean individual relative change from baseline (%): placebo 50.9; 5mg 91.2; 10mg 97.6; 25mg 83.1] [Kruskal-Wallis test p=0.002]. Mean number of bleeding days was: placebo, 11.1; 5mg, 3.5; 10mg, 1.6 and 25mg, 1.5. Endometrial morphology in the placebo group was consistent with mid/late secretory phase features. The endometrium from women treated with asoprisnil displayed features of both progesterone antagonism and agonist effects. IHC analyses demonstrated an up-regulation of ERα and PR expression in epithelial and stromal compartments in the functional and basal layers. There were no safety concerns identified.

Summary: Asoprisnil administration resulted in a rapid reduction of menstrual blood loss over a short period of treatment in women with treatment resistant HMB without organic cause. Mechanisms of treatment effect remain to be determined.

589

CALPAIN5 (CAPN5) Expression Is Regulated by HOXA10 in Human Endometrial Cells and Decidua; Aberrant Regulation in Endometriosis and Pre-Eclampsia. Ivan A Penna, Frederick Schatz,[*] Charles Lockwood,[*] Hugh S Taylor.[*] *Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA.*

OBJECTIVE: CAPN5 is member of the calpain-like cystein proteases that have been implicated in differentiation and apoptosis. We conducted a microarray screen that identified CAPN5 as target of HOXA10 transcriptional control in endometrium. Here we demonstrate regulated CAPN5 expression in endometrium, 3rd trimester decidua and demonstrate aberrant regulation in endometriosis and decidua of women with pre-eclampsia

METHODS: Ten endometrial biopsies were obtained from fertile controls. Histologically confirmed biopsies of endometriosis were obtained from 10 women at laparoscopy. Five 3rd trimester decidual samples from controls, and five 3rd trimester decidual samples from women with pre-eclampsia. Immunohistochemistry (IHC) was used to identify CAPN5 protein expression. H-SCORES were determined by 2 evaluators blinded to the study groups. The human endometrial stromal cell line, HESC,and the epithelial cell line, Ishikawa were transfected in quadruplicate with either pcDNA/HOXA10, HOXA10 siRNA or respective controls. RNA was isolated and qRT-PCR was performed. Statistical analysis was performed using Mann-Whitney test for H-SCORE and ANOVA with Bonferroni pos-test for qRT-PCR.

RESULTS: CAPN5 was expressed in endometrial stromal and glandular cells throughout the menstrual cycle. CAPN5 expression was decreased in endometriotic stromal and glandular cells to 50% of that seen in control endometrium ($p<0.05$).CAPN 5 was also expressed in decidua obtained from pre-eclamptics at higher levels than controls ($p<0.05$). The regulatory relationship between HOXA10 and CAPN5 was established by transient transfection analysis. CAPN5 gene expression increased 11-fold ($p<0.05$) after pcDNA/HOXA10 transfection of HESC, and decreased 23-fold ($p<0.05$) after HOXA10 siRNA treatment.

CONCLUSION: CAPN5 is expressed in normal endometrium, upregulated in decidua of women with pre-eclampsia and decreased in endometriosis. CAPN5 expression is regulated by HOXA10. CAPN5 is a regulatory molecule potentially involved in the pathogenesis of endometriosis and pre-eclampsia. Decreased CAPN5 in endometriosis may lead to reduced apoptosis and contribute to the increased proliferative potential of this disease; similarly increased CAILPAIN5 in pre-eclampsia, leading to increased apoptosis, may contribute to pathogenesis of this disease. (Supported by NIH36887)

590

Estrogen Receptor β (ERβ) and Progesterone Receptor-C (PR-C) Upregulation in Endometriosis May Suggest Potential Roles in Its Pathogenesis and Progression. Orhan Bukulmez,[1] Daniel B Hardy,[2] Bruce R Carr,[*1] Ruth A Word,[*1] Carole R Mendelson.[*2] *[1]Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA; [2]Biochemistry and Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA.*

Aromatase expression is enhanced in eutopical endometrium and endometriotic lesions of women with endometriosis. Induction of aromatase may be mediated by increased cyclooxygenase 2 (COX-2). Recently, we demonstrated that progesterone receptor isoforms PR-A and PR-B serve an anti-inflammatory role by antagonizing NF-κB activation and COX-2 expression. We also found that PR-C isoform, which antagonizes PR-B function, is upregulated by inflammatory mediators. While estrogen receptor α (ERα) has been suggested to mediate progression of endometriosis, a potential anti-inflammatory role of ERβ has been suggested. We, postulate that PR-C and ERβ may play opposing roles in the pathogenesis. Here, we examined the tissue- and stage-specific expression levels of ERβ relative to ERα and PR-C relative to total PR in association with aromatase and COX-2 expression in endometriosis. Biopsies of eutopical endometrium (EE), endometriosis implants (EI), visually normal peritoneum and endometrioma cyst capsules (EC) were obtained from endometriosis

patients (n=13). Endometrial and peritoneal biopsies were obtained from control women undergoing tubal sterilization (n=8). Using quantitative (q)RT-PCR and immunohistochemistry, we observed that aromatase expression in EE was increased as compared to control tissues. Aromatase expression in EI was markedly increased as compared to EE. Red implants revealed the highest aromatase expression. Importantly, expression levels of COX-2 mirrored aromatase in all tissues examined. ERβ expression in EI was higher than EE and control endometrium, while the highest levels of ERβ mRNA were detected in EC. ERβ was expressed in both stromal and epithelial components of the implants. Although expression of the three PR isoforms was detected in all tissues, the levels of PR-C relative to total PR mRNA were increased in EI and EC as compared to EE and control endometrium.We suggest that PR-C and ERβ may be upregulated by the inflammatory response associated with endometriosis. While increased PR-C may enhance the pathogenesis of this disease, upregulation of ERβ may play an anti-inflammatory and opposing role in its progression. Supported by NIH 5-R01-DK31206.

591

Molecular Profiling of the Endometrium Reveals Progesterone Resistance in Endometriosis. Richard O Burney,[1] Said Talbi,[2] Amy E Hamilton,[2] Kim Chi Vo,[2] Mette Nygaard,[2] Camran N Nezhat,[1] Bruce A Lessey,[*3] Linda C Giudice.[*2] *[1]Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA, USA; [2]Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA; [3]Reproductive Endocrinology and Infertility Division, Center for Women's Medicine, Greenville, SC, USA.*

Background: A phenotype of progesterone resistance in endometriosis is suggested by the unresponsiveness of many women to progestin-based treatment of pain associated with this disorder. An endometrium that is inhospitable to implantation in women with endometriosis may be consequent to an abnormal response to progesterone during the critical window of implantation.

Methods: Endometrial biopsies were obtained from 21 subjects with laparoscopically staged and histologically confirmed moderate-severe stage endometriosis and from 21 subjects without endometriosis. Each endometrial sample was histologically dated as proliferative, early secretory (ESE) or mid secretory (MSE), processed to biotinylated cRNA and hybridized to a GeneChip Array (HG U133 Plus 2.0, Affymetrix). Data were analyzed by bioinformatics approaches and validated using semi-quantitative real time PCR.

Results: Principal component analysis revealed molecular dysregulation of the proliferative-to-secretory transition in the endometrium of women with endometriosis. The ESE showed striking enrichment in DNA synthesis and cell cycle related genes. Coherent dysregulation of multiple genes involved in the cell cycle pathway in ESE of women with endometriosis suggested an altered response to progesterone (P)-mediated attenuation of proliferation during this phase. Differentially expressed genes possibly involved in the inadequate response to P-mediated endometrial transition include the transcription factor FOXO1A, the anti-apoptotic genes BCL2 and survivin, and members of the EGFR pathway (MIG6, TOB1, ERBB2). Expression profiling of genes known to be P-mediated in secretory phase endometrium confirmed the observation of P-resistance in endometriosis, with 34 genes showing significant dysregulation in the ESE. During the window of implantation, 15 progesterone-mediated genes, including MUC1, osteopontin and IGFBP1, were dysregulated in the MSE of women with endometriosis.

Conclusions: Collectively, these results provide compelling molecular evidence for progesterone resistance of the eutopic endometrium in women with endometriosis.

592

Differential Expression of ERbeta in Endometrial and Endometriotic Stromal Cells: Regulation by Methylation. Qing Xue, Zhihong Lin, Ping Yin, Erica Marsh, Scott Reierstad, Serdar E Bulun.[*] *OB/GYN Department, Northwestern University, Chicago, IL, USA.*

Objective: ERbeta is expressed at strikingly higher levels in endometriosis compared with eutopic endometrium. Thus, we investigated the role of methylation of the ERbeta gene promoter in differential expression of ERbeta in stromal cells of endometriosis vs. eutopic endometrium.

Methods: We used primary stromal cells in culture from ovarian endometrioma walls (n=8) and eutopic endometrium from disease-free women (n=8) to conduct methylation and gene expression studies. Transcript levels were determined by real-time PCR. DNA was demethylated using treatment with the methyltransferase inhibitor 5-Aza-CdR. Methylation status of the ERbeta gene was assessed by bisulfite modification followed by sequencing.

Results: ERalpha mRNA were somewhat lower(approximately 3-fold, $P<0.001$) in endometriotic stromal cells as compared to endometrial stromal cells, whereas ERbeta mRNA was strikingly higher(approximately 36-fold, $P<0.001$) in endometriotic stromal cells, but lower or nearly absent in endometrial stromal cells. PR-B and total PR mRNA levels in endometriotic stromal cells were also significantly lower than in endometrial stromal cells ($P<0.001$). Because the extent of differential expression was the highest for ERbeta, we pursued to determine its regulation via promoter methylation. We identified an approximately 500-bp classical CpG island (-170/+330) within the promoter regulatory region of the ERbeta gene. There was a statistically significant (t-test, $P<0.01$) difference in the methylation status within this CpG island. This region was heavily methylated in untreated endometrial stromal cells that express lower level of ERbeta and largely unmethylated in endometriotic stromal cells that express higher level of ERbeta mRNA. Treatment with the methyltransferase inhibitor 5-Aza-CdR increased ERbeta mRNA levels significantly in both endometrial stromal cells and endometriotic stromal cells (t-test, $P<0.05$).

Conclusions: This is the first demonstration of a methylation-dependent mechanism responsible for upregulation of ERbeta expression in endometriosis. Taken together, methylation status of a critical CpG island at the promoter of the ERbeta gene may be the primary regulator of ERbeta expression in endometriotic stromal cells vs. its downregulation or nearly absence in eutopic endometrial stromal cells.

Support: Grant support was provided by the NIH grant R01-HD38691.

593

Regulation of Protease Activity in Vaginal Tissues from Fibulin-5 Knockout Mice and during Normal Pregnancy, Parturition, and the Puerperium. Cecilia K Wieslander, Spyridon I Marinis, Peter G Drewes, Patrick Keller, Ruth A Word.[*] *Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA.*

Objectives: Previously, we demonstrated that a burst of elastic fiber synthesis and assembly occurs in the postpartum vaginal wall. In this investigation, we quantified the severity of prolapse in Fbln5 KO mice as a function of age and determined the expression of matrix metalloproteases in the vaginal wall of knockout and nonpregnant, pregnant and postpartum wild type mice. **Methods**: Mouse pelvic organ prolapse quantification (MOPQ) scoring was conducted on 15 WT, 61 heterozygotes, and 68 $Fbln5^{-/-}$ virginal female mice. Vaginal tissues were collected from 14 NP WT mice, 105 WT mice at various time points during pregnancy and postpartum, and 5 NP Fbln-5 KO mice with advanced stage prolapse. Expression of MMP-2, -9, and -12 was determined by real time PCR, and MMP enzyme activities were quantified using a fluorescent labeled peptide and gelatin zymography. ANOVA, Kruskal-Wallis ANOVA on ranks, and Bonferroni t-test were used to compare groups, using nulliparous nonpregnant mouse vagina as controls.**Results:** In Fbln5 knockout mice, prolapse was not present until 3-4 months of age and prolapse severity increased as a function of age such that by 6 months, > 91% experienced Stage 3 prolapse even in the absence of vaginal delivery. MMP-2 and MMP-9 mRNA increased >10-fold 12 - 24 h postpartum returning to normal levels by 48 h -1 week pp. Like mRNA levels, both pro- and active MMP-2 and MMP-9 enzyme activities were increased significantly in vaginal tissues from postpartum animals (48 h). Further, both pro- and active MMP-2 and MMP-9 enzyme activities were increased significantly in vaginal tissues from nonpregnant Fbln-5 KO mice ($P < 0.05$). **Conclusions:** The extracellular matrix of the vaginal muscularis undergoes dynamic changes during pregnancy, parturition, and the puerperium. The results are consistent with the hypothesis that continuous elastic fiber renewal is important in the supportive structures of the female pelvic floor and that increased elastase activity in the postpartum vaginal wall is counterbalanced by increased elastic fiber synthesis. The data also suggest that Fbln-5/elastic fibers play not only an important structural role, but are also important in regulation of MMP activity in connective tissues of the pelvic floor.

594

The Role of a Disintegrin and Metalloproteinase (ADAMTS-1) in USL Integrity and Pelvic Organ Prolapse. Kathleen A Connell, Marsha K Guess, Vaagn Andikyan, Hugh S Taylor.[*] *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMST-1) is a secretory protein that localizes in the extracellular matrix (ECM). It is necessary for the growth, function and structure of the female reproductive organs and is believed to participate in ECM turnover. Previous data with ADAMST-1 null mice have shown that the absence of this gene results in accelerated fibrosis and pelvic tissue deformities. We sought to evaluate the role of ADAMTS-1 in women with pelvic organ prolapse (POP).

Methods: Biopsies of the uterosacral ligament (USL) were taken at the time of hysterectomy or laparoscopic surgery from 18 women with stage $\geq$ 2 POP as defined by the Pelvic Organ Prolapse Quantification system (POP-Q). Seven women with normal pelvic support served as controls. RNA extraction was performed using Trizol. We determined ADAMTS-1, collagen type I and type III mRNA expression using quantitative real time RT-PCR. Beta actin was used as an internal control.
Results: ADAMTS-1 expression was decreased ten-fold in USL of women with POP compared to controls (p=0.01, n= 13). No changes were seen in collagen types I and III (p= 0.79, and p=0.55,respectively, n=25).
Conclusion: ADAMTS-1 is a newly identified protein involved in ECM metabolism and female reproductive organ development. In women with POP, expression of ADAMST-1 in the USL was found to be significantly reduced. Accelerated fibrosis would be expected following downregualtion of ADAMTS-1. Our findings of decreased ADAMTS-1 in the USL suggest that loss of this protein may contribute to abnormal USL integrity in POP.

595

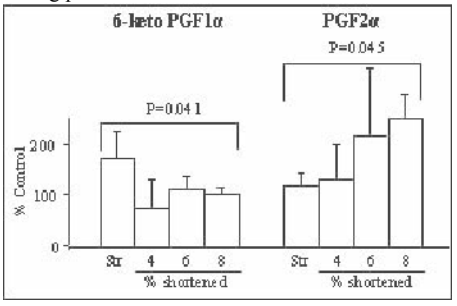
Adolescent Obesity Exerts a Detrimental Effect on Lifetime Parity Independent of Adult Body Mass: Baseline Data from the Study of Women's Health across the Nation. Alex J Polotsky,[1] Susan Hailpern,[1] Joan Skurnick,[2] Joan Lo,[3] Barbara Sternfeld,[3] Nanette Santoro.*[1] *[1]Albert Einstein College of Medicine, Bronx, NY; [2]UMDNJ – New Jersey Medical School, Newark, NJ; [3]Kaiser Permanente, Oakland, CA.*
Methods: The Study of Women's Health Across the Nation (SWAN), a multi-site study of women as they transition from pre- to post-menopause, collected information at baseline on self-reported childbearing and high school weight. High school BMI was categorized according to the World Health Organization (WHO) categories: <18.5, 18.5-24.9, 25-29.9, and ≥30 kg/m2. Adolescent overweight status was defined as a high school BMI ≥ 25.0 kg/m2. Multivariate logistic regression models assessed association between adolescent overweight status and lifetime nulliparity (as dichotomous outcome of any live birth vs. none) while adjusting for adult BMI at baseline, marital status, smoking, race, socio-economic status, education, andstudy site. Separate sensitivity analyses were performed by excluding participants within the lowest and highest adolescent BMI categories or excluding those who reported "sexual intercourse with women only" & "never tried to get pregnant". A secondary analysis was performed to assess the association between adolescent overweight and nulligravid status (any pregnancy vs. none). Participants who reported tubal or male infertility were excluded from analysis.
Results: Among the SWAN cohort (n=3120, mean age at baseline 45.8±2.7), the proportion of women with at least one live birth was 83.1% and showed a significant downward trend across the WHO categories of adolescent BMI: 86%, 83.4%, 81.0%, 66.3%, respectively (p<0.001). Multiple logistic regression demonstrated that adolescent overweight status was independently associated with lifetime nulliparity (OR: 1.73; 95% CI: 1.19-2.54; p=0.004). Notably, adult BMI was not associated with nulliparity in the multivariate analysis (OR =1.0, p= 0.35). Sensitivity analyses did not significantly change the results. When nulligravid status was used as the outcome variable, adolescent overweight status was confirmed as an independent risk factor for no history of pregnancy (OR: 2.21; 95% CI: 1.45 -3.36; p<0.001).
Conclusions: In the SWAN cohort, being overweight as an adolescent is associated with reduced lifetime parity and gravidity irrespective of adult BMI.
SWAN is supported by the National Institutes of Health (NR004061, AG012505, AG012535, AG012531, AG012539, AG012546, AG012553, AG012554, AG012495).

596

Effects of Relaxin on Cyclooxygenases in Human Maternal-Fetal Membranes and Myometrial Cells. Dunja M Baston,[1] Jens Hirchenhain,[1] Jan-Steffen Kruessel,[1] Ulrike Friebe-Hoffmann,[1] Phillip N Rauk.*[2] *[1]Department of OB/GYN, University of Duesseldorf, Duesseldorf, NRW, Germany; [2]Department of OB/GYN, University of Minnesota, Minneapolis, MN, USA.*
Introduction: 5-10 % of all newborn children in the industrial nations are prematurely delivered before 37 weeks of gestation. Oxytocin (OT) and its corresponding receptor (OTR) play a key role in an autocrine/paracrine system regulating myometrial contractions. During pregnancy relaxin (RLX), a polypeptide hormone produced in the *corpus luteum* of pregnancy, placenta as well as decidua, causes significant myometrial relaxation in pregnant rodents by inhibiting PIP_2 turnover and therewith OTR signalling via prostaglandin synthesis. Even after clinical studies its role in of uterine contractions in humans still remains unclear. In former studies we could show an inhibition of OTR mRNA and protein expression in human myometrial cells after incubation with RLX. By showing that IL-1ß downregulates OTR in human myometrial cells while stimulating the cyclooxygenase (COX) system and therewith contractile prostaglandins, the purpose of the present study was to investigate RLX´s influence on cyclooxygenase-1 and -2 (COX) in human myometrial and decidual cells.
Methods: Primary cultures of human myometrium and decidua (n=8) from pregnant women undergoing elective caesarean section at term were incubated with different concentrations of RLX [0-100 ng/ml], infection-associated molecules (LPS, cytokines), OT, COX inhibitors and their combinations for different time periods [0-24 h]. The expression of COX-1 and COX-2 was measured by Western blot and real-time PCR.
Results: RLX significantly stimulated COX-1 and COX-2 expression on mRNA and protein levels more than 2-fold leading to an enhancement of contractile prostaglandins.
Conclusion: This is the first time an effect of RLX on the COX system is described in human cells of pregnant myometrium and decidua. This contraction supporting effect already seen in former studies with IL-1ß stimulation stands in controversy to RLX triggered down regulation of the OTR showing once more the complexity of intracellular mechanisms leading to uterine contractions and birth.

597

Myometrial Prostaglandin Production Is Modulated by Changes in Length. William W Hurd,* Shawn G Gibbs, Kari A Rudinski. *Obstetrics and Gynecology, Wright State University, Dayton, OH, USA.*
Objective: To determine if stretching and subsequent shortening of myometrial strips from pregnant women alters production of prostaglandins $PGF_{2\alpha}$ and PGI_2.
Methods: The concentrations of $PGF_{2\alpha}$ and 6-keto$PGF_{1\alpha}$ (a stable metabolite of PGI_2) were measured in the contraction baths of 50 myometrial strips obtained at cesarean delivery from 5 pregnant women at term not in labor. Fluid was collected after 2 hours of stretching, and after the strip lengths were decreased by 4%, 6%, or 8%. Control strips were maintained without tension throughout the experiments. PG concentration (pg/mg tissue) was measured for each strip in triplicate by competitive enzyme immunoassay and compared by Kruskal-Wallis analysis.
Results: Stretching myometrial strips (Str) increased production of 6-Keto$PG_{1\alpha}$ by 76%, but had little effect on $PGF_{2\alpha}$. Shortening strips had little effect on 6-Keto $PGF_{1\alpha}$ production, but increased production of $PGF_{2\alpha}$ up to 158%.
Conclusion: Stretching myometrial strips from pregnant women at term increases synthesis of PGI_2 while shortening strips increases synthesis of $PGF_{2\alpha}$. Differential PG synthesis related to myometrial stretching and shortening could explain in part uterine quiescence during gestation and increased contractility during parturition.



598

Decreased Expression of Prostaglandin E2 Receptor Isoforms EP3-II, III, and VI in Lower Segment Human Myometrium Is Greater with Induced Labor Than in Spontaneous Labor. Richard H Lee, Thomas M Goodwin,* Patrick M Mullin, Aimin Li, Gloria Yang, Juan C Felix. *Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA.*
OBJECTIVE: To assess relative quantification of prostaglanidin E2 receptor isoforms EP3-II, III, and VI in lower uterine segment myometrium in the non-pregnant state and three states of term pregnancy: non-labor, spontaneous labor, and induced labor.
STUDY DESIGN: Lower uterine segment myometrial samples were obtained at time of cesarean delivery (n=18) or hysterectomy (n=9). Labor was defined as regular uterine contractions resulting in cervical change. Parturients were stratified as either having spontaneous labor (n=4) or induced labor (n=5) based

on the use of labor induction agent (pitocin=1, foley=2, dinoprostone insert=2). Relative quantification for EP3 isoforms II, III, and VI was performed using real-time RT-PCR. Stastistical analysis was performed using non-parametric tests.

RESULTS: All three EP3 isoforms were expressed in lower uterine segment myometrium. There were differences in all three EP3 isoforms amongst the four groups (EP3-II, *P*=0.006, EP3-III, *P*=0.033, EP3-VI, *P*=0.006). There was a significant increase in EP3-VI expression in non-laboring pregnant tissue when compared to the non-pregnant state (11.9 fold, *P*=0.024). When compared to spontaneous labor, non-laboring pregnant subjects had significantly increased expression in EP3-II (3.8 fold, *P*=0.005) and EP3-VI (5.3 fold, *P*=0.020). Non-laboring subjects demonstrated significantly increased levels of all three isoforms when compared to induced subjects [EP3-II (9.3 fold, *P*=0.003), EP3-III (5.6 fold, *P*=0.004), EP3-VI (43.3 fold, *P*=0.003). Spontaneously laboring subjects also demonstrated significantly increased levels of all three isoforms when compared to induced subjects [EP3-II (2.5 fold (*P*=0.014), EP3-III (3.0 fold (*P*=0.05), EP3-VI (8.1 fold (*P*=0.05).

CONCLUSIONS: In human parturition, lower-uterine segment EP3 receptor isoforms-II, III, and VI are involved in maintaining lower uterine segment tone. Decreased expression of these isoforms is common to both induced and spontaneous labor. However, the profound decrease in EP3 gene expression in induced labor suggests modification in EP3 receptor activity is more critical in this pathway to parturition.

599

Effects of RU486 and Progesterone Supplementation on Prostaglandin Receptor Expression in Pregnant Rat Uterus and Cervix. Peta L Grigsby, Andy C Hinton, Brad A Pitzer, Diane E Brockman, Leslie Myatt.* *OB/GYN, Uni of Cincinnati, Cincinnati, OH, USA.*

Introduction: Prostaglandin E2 (PGE2) appears to play a role in the biochemical and structural changes that facilitate uterine activation and stimulation and cervical ripening for labor. The effects of PGE2 are mediated through specific G protein coupled contractile and relaxatory receptors (EP1-4). The hormonal mechansims that regulate the expression of these receptors in the uterus and cervix are yet to be elucidated, but may involve the steroid hormone progesterone. The aim of this study was to determine the expression of the PGE_2 receptor isoforms in pregnant rat uterus and cervix during RU486 induced labor and with progesterone supplementation following ovariectomy.

Methods: Labor was induced in pregnant rats by injection with RU486 (10mg/kg IP; n=5) or vehicle (n=6) on day 16 of gestation. A second group were ovariectomized (OVX) on day 16 and subsequently received daily injections of progesterone (P4, 2.5mg/day SQ; n=5) for 48 h. A surgical control group was included (n=5). Uteri (myo+endo) and cervical tissues were collected on day 18 of gestation. Total RNA was extracted from the uterus and cervical samples using the Trizol® method. Quatitative real-time PCR for EP1-4 was performed using Taqman® Gene Expression Assay kits. Target gene mRNA abundance was calculated from a standard curve and normalized to βactin.

Results: There were no significant differences in EP1-4 expression in the uterus associated with RU486 induced labor. However, EP1 and EP2 mRNA expression increased in the cervix after RU486 treatment, while EP3 and EP4 remained unchanged. OVX plus P4 supplement significantly increased EP1, EP2 and EP4 in the uterus, with no effect on EP3. In contrast, OVX plus P4 treatment upregulated the expression of EP3 in the cervix, yet had no effect on EP1, EP2 or EP4 levels.

Conclusions: PGE2 receptor expression in the uterus did not change after RU486 induced labor, whereas, cervical EP1 and EP2 were elevated. The upregulation of relaxatory receptors in the uterus after P4 treatment is consistent with a quiescent phenotype during pregnancy to accommodate the growing fetus. Additionally, increased EP3 may aid the cervix in remaining tightly closed during this time. The lack of coordination in receptor expression between the uterus and cervix with labor and after P4 treatment may indicate differential regulatory mechanisms associated with the PGE2 receptors between these tissues.

600

Expression of Prostaglandin E_2 Receptors in Human Pregnant Myometrium during Pregnancy. Mandeep S Kandola,[1] Shankari Arulkumaran,[1] Richard J Wilson,[2] Bryan E Hoffman,[2] David P Brooks,[2] Phillip R Bennett.*[1] *[1]Parturtion Research Group, Imperial College, London, United Kingdom; [2]GlaxoSmithKline, Harlow/King of Prussia, United Kingdom.*

Inhibition of the action of prostaglandin E2 may be a fruitful strategy in preventing preterm delivery. We have therefore studied the expression pattern of the prostaglandin E2 receptors EP-1, -2, -3 and -4 in human upper and lower segment myometrium at term.

Immunohistochemical studies were undertaken to compare the expression of EPs between upper and lower segment myometrium taken before and after labour. Positive controls were CHO cells transfected with expression vectors for each EP.

Immunohistochemistry did not show any significant differences in expression of EPs between upper and lower segment. Immunohistochemistry demonstrated expression of EP1 was principally cytoplasmic prelabour, but nuclear post-labour. Western blot analysis showed no overall changes in expression of EP1. Immunohistochemistry showed a marked reduction in expression and nuclear localization of EP2 in post-labour, compared to pre-labour samples. Western blot analysis EP-2 was detected in pre-labour but not in post-labour samples. There are nine known isoforms of EP-3. RT-PCR using primers specific for each isoform showed expression for all in both upper and lower segment. Expression of EP3-V, EP3-VII and EP3-IX at mRNA level increased in post-labour tissues by 2- 5- and 6-fold respectively. Immunohistochemistry showed a marked increase in EP-3 expression in both upper and lower segments with expression remaining cytoplasmic post-labour. Western blot analysis demonstrated an increase in expression of EP3-V with labour, and high levels of EP3-VII and EP3-IX both pre and post-labour.

Immunohistochemistry showed a tendency for decreased expression of EP-4 associated with labour. Western analysis of EP-4 showed no differences in overall expression.

In organ bath studies an EP-3 receptor antagonist led to a 2 fold reduction in spontaneous myometrial contractility, and inhibited the increase in contractility stimulated by PGE2.

These data suggest that, as part of the change of the myometrium to a contractile phenotype there is down regulation of the relaxatory EP-2 and EP-4 and that the principal EP mediating the action of exogenous PGE2 in contracting myometrium is EP3. The role of nuclear translocation of EP-1 requires further study. Taken together these data suggest that antagonists of EP-3 may be useful in prevention of uterine contractions.

601

The Role of Prostaglandin E_2 Receptors in Human Myometrium during Pregnancy. Shankari Arulkumaran,[1] Mandeep Kandola,[1] Richard J Wilson,[2] David P Brooks,[3] Phillip R Bennett.*[1] *[1]Imperial College Parturition Research Group, IRDB Hammersmith Hospital Campus, London, United Kingdom; [2]Rheumatoid Arthritis Biology, GlaxoSmithKline, Stevenage, Hertfordshire, United Kingdom; [3]Cardiovascular and Urogenital Diseases CEDD, GlaxoSmithKline, King of Prussia, PA, USA.*

Human myometrial strips established in culture will usually begin contracting after approximately one hour. The mechanism for this is poorly understood. We have previously shown that stretch upregulates prostaglandin synthesis and have therefore hypothesized that spontaneous contractions occur because of stretch -related prostaglandin synthesis.

Experiments were performed using 5x2 mm human pre-labour, lower uterine segment myometrial strips in a DMT Myograph 800MS in oxygenated Kreb's solution, with ADI Powerlab software. In initial experiments, myometrial strips were subjected to stretch force between 0 and 12g. Spontaneous contractions required stretch force. The number of strips attaining spontaneous contractions was greatest at a force of 5-6g. Increasing tension above 6g reduced spontaneous contractility.

Incubation of strips with acetyl salicylic acid (ASA) an inhibitor of both COX-1 and COX-2 for one hour prior to the experiment led to a 3-fold reduction in peak tension and total work done. Incubation of ASA pre-treated strips with PGE_2 (at any concentration between 10^{-10} to 10^{-6}) returned peak tension and overall work done per contraction to normal values, but also increased contraction rate, leading to an overall increase in total work done when compared to (ASA and PGE_2 non-treated) controls.

Addition of the EP-3 antagonist L-798106 prior to the experiment led to a 2-fold reduction in peak tension and therefore a reduction in total work done. Furthermore, incubation of EP-3 antagonist L-798106 pre-treated strips with PGE_2 (at any concentration between 10^{-10} to 10^{-6}) inhibited peak tension and overall work done per contraction similar to that in non-treated controls.
These data suggest that stretch and synthesis of prostaglandins is essential for spontaneous contractility in human myometrial strips. Since the reduction in contractility caused by ASA was greater than that caused by L-798106, it is likely that prostaglandin E2 plays an important but not exclusive role. However, it does show that PGE_2 acts predominantly via EP-3 receptors, thus providing support for the concept that antagonism of the EP-3 receptor may be a suitable strategy for pharmacological tocolysis.

602

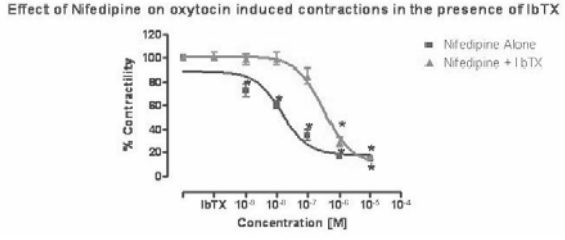
Progesterone Is Required for the Maintenance of Relaxin Receptors (Lgr7) in the Myometrium of Late Pregnant Rats. Lenka A Vodstrcil,[1,2] Laura J Parry,[1] Stephen J Lye,[*3] Mary E Wlodek,[*2] Oksana Shynlova.[3] *[1]Zoology, Univ of Melbourne, Melbourne, VIC, Australia; [2]Physiology, Univ of Melbourne, Melbourne, VIC, Australia; [3]Samuel Lunenfeld Res Institute, Mt Sinai Hospital, Toronto, ON, Canada.*
Serum levels of the ovarian hormone relaxin are elevated during the second half of rat pregnancy. Relaxin as well as progesterone (P4) may be required to maintain myometrial quiescence in pregnant rodents. The actions of relaxin are mediated by a leucine-rich repeat containing G-protein coupled receptor, Lgr7 (RXFP1). Myometrial Lgr7 gene expression is highest in early- to mid-gestation in the rat and decreases at term. The factors that regulate Lgr7 are poorly understood. Progestin, in the presence or absence of relaxin, stimulates Lgr7 expression in human endometrial stromal cells. Maternal P4 levels peak between days 15-19 in pregnant rats and then decrease at term. Therefore, we hypothesized that blocking the action of P4 in late pregnancy will decrease Lgr7 in the myometrium. Conversely, progesterone administration will produce the opposite effect.
OBJECTIVE: This study tested the effect of early progesterone withdrawal and progesterone administration (to maintain plasma hormone levels) on the expression of Lgr7 in the myometrium.
METHODS: Rats were treated with the P4 receptor antagonist, RU486, or vehicle on day 19 of gestation and myometrial tissue was collected 24 h later. Pregnant rats received daily subcutaneous injections of either P4 or vehicle starting on day 20 gestation. Myometrial tissue was collected on days 21, 22 or 23 (during labor) in the vehicle-treated group or days 21, 22, 23, or 24 in the P4-treated group. Total RNA was extracted from the myometrial samples and analyzed using quantitative real-time PCR.
RESULTS: Administration of RU486 on day 19 caused the onset of preterm labor within 24 h and resulted in a significant decrease in myometrial Lgr7 gene expression. In contrast, daily injections of exogenous P4 had no significant effect on Lgr7 expression.
CONCLUSIONS: Treatment with RU486, to induce preterm withdrawal of P4, down-regulated Lgr7 gene expression in the myometrium. This demonstrates an important role for progesterone in maintaining Lgr7 expression in late pregnant rats, thereby preventing premature onset of delivery. Prolonging the length of gestation with progesterone had no significant effect on Lgr7 expression. This suggests that the decrease in Lgr7 at term is due to progesterone-independent mechanisms.

603

The Role of Protein Kinase C in Mediating Responsiveness to Oxytocin in Uterine Strips from Pregnant and Non-Pregnant Rats. Patrice L Arthur,[1] Bryan F Mitchell,[*2] Michael J Taggart.[1] *[1]Maternal and Fetal Health, St. Mary's Hospital, University of Manchester, Manchester, United Kingdom; [2]Department of Obstetrics and Gyneacology, University of Alberta, Edmonton, AB, Canada.*
Background: Stimulation of uterine activity is modulated by G-protein coupled receptors such as the oxytocin (OT) receptor (OTR). These receptors are coupled to membrane phospholipase C and activate the inositol trisphosphate and protein kinase C (PKC) pathways. We hypothesized that manipulation of PKC activity would influence uterine activity in the rat. Further, we hypothesized that the role of PKC in regulation of uterine contractility would change at the time of uterine activation.
Methods: Uterine muscle strips were obtained from non-pregnant (NP) and pregnant Sprague Dawley rats during relative uterine quiescence (d18) and at term just prior to labour onset (d21). Quantitative real time RT-PCR was used to assess mRNA for OTR and FP markers of uterine activation in the day 21 samples. Contractile responses to OT (5 nmol/L) were assessed using myography before and after in vitro treatment with the PKC agonist, 12,13-phorbol dibutyrate (PDBu; 10^{-9} to 10^{-6} mol/L) or the antagonist bisindolylmaleimide (Bis-1: 10^{-9} to 10^{-6} mol/L). The effects of the PKC agonist or antagonist were calculated as the percentage of the contractile activity in the presence of the drug compared to the pre-treatment control.
Results: PDBu-treated strips showed a concentration-dependent and significant decrease in uterine contractility. Maximal suppression was to 34.1 ± 11.4% in the NP group (mean ± SEM; n=5), 10.3 ± 6% in the d18 animals (n=5) and to 12.3 ± 12.5% in the d21 group (n=5; all significant at $P \leq 0.01$) compared to control. Maximal inhibition in all groups occurred at 1 uM (2-way ANOVA and post-hoc Bonferroni). There was no significant change in uterine contractility in response to OT in strips that were treated with BIS. The uterine mRNA levels for OTR and FP significantly increased ($P<0.05$) from d18 to d21 (1.7 ± .06 to 18.1 ± 5.9 and 2.0 ± 0.4 to 8.7 ± 2.4 respectively).
Conclusion: These data suggest a role for PKC isotypes, once activated, in limiting OT responsiveness in rat myometrium. Since the PKC inhibitor BIS had no effect in any of the animals we suggest that there was no basal PKC-mediated mechanism influencing the uterine response to OT. Future studies using more specific PKC isotype inhibitors will elucidate in detail the role of PKC in OT responsiveness.

604

Effects of Potassium Channel Blockade on Nifedipine-Induced Relaxation of Pregnant Human Myometrial Contractility. Audrey T Moynihan,[1,2] Terry J Smith,[2] John J Morrison.[*1] *[1]Obstetrics and Gynaecology, National University of Ireland, Galway, Galway, Ireland; [2]National Centre for Biomemdical Engineering Science, National University of Ireland, Galway, Galway, Ireland.*
OBJECTIVE: Potassium channels are key components involved in regulating the levels of intracellular calcium in the myometrium, and therefore present themselves as key targets for tocolytic therapy for the treatment and prevention of preterm labour. The aim of this study was to investigate the roles of TEA and IbTX on the effects of nifedipine in pregnant non-labouring human myometrium to ascertain if the inhibition of smooth muscle contraction by nifedipine was mediated wholly or in part by the BK_{Ca} channel.
METHODS: Biopsies of human myometrium were obtained at elective cesarean section (n=24). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous and oxytocin-induced contractions, were exposed to either TEA/IbTX followed by cumulative additions of nifedipine (1 nmol/L - 10 µmol/L). Control strips were run simultaneously. Integrals of contractile activity were measured using the PowerLab hardware unit and Chart v3.6 software. Data were analysed using one-way ANOVA followed by post hoc analysis.
RESULTS: Nifedipine exerted a potent and cumulative inhibitory effect on oxytocin-induced contractions in human myometrium *in vitro*, in comparison to control measurements ($P< 0.05$, n=6). Incubation of strips with TEA or IbTX, prior to addition of nifedipine significantly attenuated the relaxant effect exerted by nifedipine ($P<0.05$, n=6) at bath concentrations of 1 nmol/L, 10 nmol/L, 100 nmol/L and 1 µmol/L ($P < 0.05$). Similar results were obtained in the spontaneous model, where both TEA and IbTX had a significantly antagonistic effect on nifedipine-induced relaxation of the myometrium.



CONCLUSIONS: This study has shown for the first time that nifedipine acts through the BK_{Ca} channel. This suggests that K^+ channel conductance and particularly the BK_{Ca} channel plays a role in the potent relaxant effect of nifedipine, hitherto presumed to act solely through L-gated calcium channels.

SATURDAY

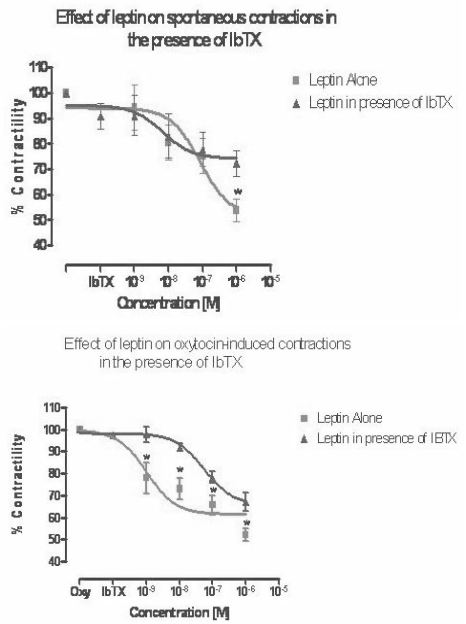
605

Leptin and the BK_{Ca}: Effects on Human Uterine Contractility *In Vitro*. Audrey T Moynihan,[1,2] Terry J Smith,[2] John J Morrison.[*1] *[1]Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Galway, Ireland; [2]National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland.*

OBJECTIVE: We have previously reported that leptin significantly inhibits uterine contractility *in vitro,* however the exact mechanism by which leptin inhibits contractility is unknown. Studies in hippocampal neurons of the brain have shown that leptin inhibits rat hippocampal neurons by activating the BK_{Ca} channel. We hypothesized that leptin significantly inhibits uterine contractility by activating the BK_{Ca} channel in human myometrium.

METHODS: Biopsies of human myometrium were obtained at elective cesarean section (n=18). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous or oxytocin-induced contractions, were exposed to IbTX followed by cumulative additions of leptin (1 nmol/L - 1 µmol/L). Control strips were run simultaneously. Integrals of contractile activity were measured using the PowerLab hardware unit and Chart v3.6 software. Data were analysed using one-way ANOVA followed by post hoc analysis.

RESULTS: Leptin exerted an inhibitory effect on spontaneous and oxytocin-induced contractions in human myometrium *in vitro*, in comparison to control measurements. The effect of leptin was significantly antagonized by IbTX at bath concentrations of 1 µmol/L ($P<0.05$) for spontaneous contractions and at bath concentrations of 1 nmol/L, 10 n mol/L, 100nmol/L and 1 µmol/L ($P < 0.05$) for oxytocin-induced contractions.



CONCLUSIONS: This study has demonstrated that leptin acts through the BK_{Ca} channel in smooth muscle myometrium.

606

Local and Systemic Regulation of the Relaxin Receptor (Lgr7) in the Myometrium of Pregnant Rats. Lenka A Vodstrcil,[1,2] Laura J Parry,[1] Stephen J Lye,[*3] Mary E Wlodek,[*2] Oksana Shynlova.[3] *[1]Zoology, Univ of Melbourne, Melbourne, VIC, Australia; [2]Physiology, Univ of Melbourne, Melbourne, VIC, Australia; [3]Samuel Lunenfeld Res Institute, Mt Sinai Hospital, Toronto, ON, Canada.*

During pregnancy, the peptide hormone relaxin is thought to contribute to the maintenance of uterine quiescence by reducing the contractile activity of myometrial smooth muscle cells. The relaxin receptor is a leucine-rich repeat containing G-protein coupled receptor, Lgr7 (RXFP1). In mice, Lgr7 is localized to the myometrium, with a significant decrease in Lgr7 expression at term. Recent reports also suggest that the ligand itself may down-regulate its own receptor in the murine uterus. To date, no physiological studies have investigated the potential effects of the fetal-placental unit on Lgr7 expression in the myometrium.

OBJECTIVE: To establish the temporal pattern of myometrial Lgr7 gene expression in rats throughout gestation, and to investigate the influence of the presence or absence of fetal-placental units on these receptors by comparing the gravid and non-gravid horns of unilaterally pregnant rats.

METHODS: Myometrium was collected from pregnant Wistar Kyoto rats at different stages of gestation, as well as post-partum and non-pregnant rats. The second experiment used myometrium collected mid- to late- gestation from rats that had undergone unilateral ovarian tubal ligation prior to mating to isolate both gravid and empty uterine horns. Total RNA was isolated for analysis using quantitative real-time PCR.

RESULTS: Myometrial Lgr7 gene expression did not change in early- to mid- gestation but was significantly reduced in late pregnancy between days 21-23. After birth, there was a dramatic increase in Lgr7 so that receptor expression returned to non-pregnant levels. Analysis of myometrium from unilaterally pregnant rats demonstrated no change in Lgr7 gene expression in the gravid myometrium compared with the normal gestational profile. But there was an increase in Lgr7 gene expression in the non-gravid horn at all stages examined.

CONCLUSIONS: These data show a down-regulation in myometrial Lgr7 gene expression in late pregnancy, which coincides with high circulating levels of relaxin. The decrease in myometrial Lgr7 may be necessary to reduce smooth muscle responsiveness to circulating relaxin, thereby activating contractile-related proteins. The decrease in Lgr7 is regulated, in part, by the presence of fetal-placental units whether through endocrine and/or stretch mechanisms.

607

The Role of the Rho-Associated Kinase in Mediating Responsiveness to Oxytocin in Uterine Strips from Pregnant and Non-Pregnant Rats. Patrice L Arthur,[1] Bryan F Mitchell,[*2] Michael J Taggart.[1] *[1]Maternal and Fetal Health, St. Mary's Hospital, University of Manchester, Manchester, United Kingdom; [2]Department of Obstetrics and Gynaecology, University of Alberta, Edmonton, AB, Canada.*

Background: G-protein coupled receptor agonists such as oxytocin (OT) can stimulate contractile activity through the phospholipase C - inositol trisphosphate pathways. However, they can also increase the force generated by a given Ca^{2+} concentration through a process referred to as Ca^{2+}-sensitization. The Rho-associated kinase (ROK) pathway is an important mediator of Ca^{2+}-sensitization in vascular smooth muscle. We have hypothesized that ROK plays a role in regulating myometrial contractility and that this process is augmented at the time of uterine activation.

Methods: Uterine muscle strips were obtained from non-pregnant (NP) and from pregnant rats during relative uterine quiescence (d18) and after uterine activation (d21). Quantitative real time RT-PCR was used to assess mRNA for OTR and FP markers of uterine activation. Uterine contractile responses to OT (5 nmol/L) were assessed using myography before and after in vitro treatment with the ROK antagonist H-1152 10^{-7} to 10^{-5} mol/L. The effect of this drug was calculated as the percentage of the contractile activity in the presence of the drug compared to the pre-treatment control.

Results: H-1152 caused a concentration-dependent and significant ($P< 0.05$), suppression of the response to OT in the NP (to 26.1 ± 3.5%; n=5), d18 (to 61.32 ±3.8 %; n=5) and in the d21 group (to 42.36 ± 12%; n=5) compared to control. There was a significant gestation-dependent effect only between the NP and d18 samples (multiple ANOVA and post-hoc Bonferroni). The uterine mRNA levels for OTR and FP significantly increased ($P<0.05$) from d18 to d21.

Conclusion: The ROK antagonist H-1152 caused a significant decrease in uterine response to OT in all groups. However, there appeared to be reduced ROK activity in the samples obtained at d18 compared to NP. This may suggest that the components of the ROK pathway are down-regulated, or blocked by an intrinsic inhibitor within the myometrium. We suggest that the diminished activity of the ROK pathway has important implications in helping to maintain uterine quiescence through pregnancy. Surprisingly restoration of Ca^{2+}-sensitization through the ROK pathway did not seem to occur at d21. Further experiments with animals in spontaneous labour may elucidate the role of ROK at this time.

608

Expression of α5 Integrin in Human Myometrium during Late Pregnancy and Labour. Margaret O'Brien,[1] John J Morrison,[*1,2] Terry J Smith.[2] *[1]National Centre for Biomedical and Engineering Science, National University of Ireland, Galway, Galway, Ireland; [2]Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Galway, Ireland.*

Introduction: Substantial remodelling of the extracellular matrix (ECM) occurs during late pregnancy where Type IV collagen, laminin, and fibronectin are deposited around smooth muscle cells in myometrium. Fibronectin mRNA and protein expression increased in the myometrium before labour (Nishinaka and Fukuda 1991; Shynlova et al., 2004). The interaction of fibronectin with

its major receptor, α5ß1 integrin, is important for fibronectin matrix assembly and strong intercellular cohesion. Integrin receptors are integral components of focal adhesions. These ECM-binding, heterodimeric, transmembrane receptors are composed of α and ß subunits, and the composition of some of these heterodimeric receptors is very specific, for example, α5 integrin partners solely with ß1 integrin in cell membranes to form a fibronectin receptor. The mRNA expression of α5 integrin increased throughout quiescence in rat myometrium and a decrease in expression was then observed at labour (relative to late pregnancy), and a further decrease was observed 1 day postpartum (Williams et al., 2005). While integrins have been reported to be expressed in nonpregnant human myometrium no data is available on human myometrial integrin expression during pregnancy and labour.

Methods: Myometrial biopsies were obtained at elective and emergency caesarean section and snap-frozen in liquid nitrogen. Following RNA (n=6 pregnant at-term and n=6 labouring human myometrium) and protein isolation (n=3 pregnant at-term and n=3 labouring myometrium), real time RT-PCR and western blotting were performed.

Results: Real time RT-PCR and western analysis demonstrated expression of α5 integrin in the both the pregnant at-term and labouring human myometrium. The expression of α5 Integrin mRNA significantly decreased (4-fold, p=0.000) in the labouring myometrium samples in comparison to the pregnant at term samples. α5 Integrin protein expression also decreased in the human myometrium biopsies at labour.

Conclusion: α5 integrin expression was observed in human pregnant and labouring myometrium. The expression of α5 Integrin mRNA and protein decreased at labour in comparison to the pregnant at-term human myometrium. α5 integrin expression may decrease after myometrial contractions occur at labour, when uterine growth is no longer necessary.

609

Upregulation of PAR-1 and PAR-4 Expression in Human Myometrium at Labour. Margaret O'Brien,[1] John J Morrison,[*1,2] Terry J Smith.[1] [1]*National Centre for Biomedical and Engineering Science, National University of Ireland, Galway, Galway, Ireland;* [2]*Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Galway, Ireland.*

Objective: The myometrium is transformed from a state of relative quiescence during pregnancy, to one of maximal contractile activity at the time of labour. Thrombin has been reported to stimulate myometrial contractility (Elovitz et al., 2000) through its' interaction with the protease activated receptor, PAR-1 (O'Sullivan et al., 2004). It also interacts with its other receptors, PAR-2, 3 and 4 in other tissue including smooth muscle. Thrombin is activated from the inactive zymogen, prothombin and converts fibrinogen to fibrin during blood clotting. There is an increase in prothrombin fragments and thrombin anti-thrombin complexes during pregnancy (Brenner et al., 2004). We have previously reported the expression of PAR-1, 2, 3 and 4 in human myometrium (O'Brien et al., 2004). PAR-1 expression increased 10 fold in the rat during pregnancy relative to the non-pregnant state (Shintani et al., 2000). The aim of this study was to investigate the expression of prothrombin, fibrinogen and the quantitative expression of PAR 1 and 4, in pregnant at-term and labouring human myometrium.

Methods: Myometrial biopsies were obtained at elective and emergency caesarean section and snap-frozen in liquid nitrogen. Following RNA (n=6 pregnant at-term, n=6 labouring human myometrium) and protein isolation (n=3 pregnant at-term, n=3 labouring human myometrium), real time RT-PCR and western blotting were performed.

Results: PAR-4 mRNA expression significantly increased (10-fold, p=0.022) in the labouring myometrium samples in comparison to the pregnant at term samples. PAR-1 mRNA expression also increased (8.96 fold), though not significantly, (p=0.054). PAR-1 and PAR-4 protein expression increased in the 3 labouring samples relative to the pregnant at-term myometrial samples. Fibrinogen expression was observed during pregnancy and labour, with α and β being the predominant isoforms. Prothrombin expression was also evident by RT-PCR and western blotting during pregnancy and labour although there was no significant difference in prothrombin protein expression between the two gestation states.

Conclusion: Prothrombin and fibrinogen expression was observed during pregnancy and labour. PAR-1 and PAR-4 mRNA and protein expression increased at labour suggesting a role for these thrombin receptors in human myometrium at labour.

610

Stimulation of Contractions in Human Myometrium by Serotonin Is Unmasked by Forskolin. Yolande Cordeaux, Hannah Missfelder-Lobos, D Stephen Charnock-Jones,[*] Gordon CS Smith.[*] *Obstetrics and Gynaecology, University of Cambridge, United Kingdom.*

Introduction: Several lines of evidence suggest that 5-HT may be important in controlling uterine contraction. Few studies have addressed the effects of 5-HT or selective agonists on human myometrium. Studies in vascular smooth muscle have demonstrated that in order to observe some of the effects of 5-HT, activation of adenylyl cyclase is required. There have been no such studies in human myometrium. We have therefore investigated the effect of 5-HT in this tissue, in the presence and absence of forskolin.

Methods: This study was approved by the Local Ethics Committee. All participants gave their informed written consent. Myometrial biopsies were taken from non-labouring patients undergoing elective cesarean section. Myometrial strips were stimulated with 50mM KCl and then incubated in Kreb's solution containing vehicle or 1μM forskolin. Strips were incubated with increasing doses of 5-HT (10nM to 10μM), every 20mins. Isometric tension was recorded, and "area under the curve" (AUC) calculated, using Powerlab® software Chart 5.2.2

Results: A reduction in contractions occurred within 20mins of forskolin addition. Compared to contractions in the preceding 20mins, AUC values were reduced to 51±5% (mean±s.e.) in forskolin-treated strips, but were 86±4% (mean±s.e.) in control strips. In the absence of forskolin, 5-HT did not significantly increase contractions (AUC value with 10μM 5-HT = 0±14% over basal, n=6, P=0.57, paired t-test). In the presence of forskolin, 5-HT caused a significant increase in contractions (pEC_{50}= 7.04±0.17, n=7). AUC values at the maximally effective dose of 5-HT were 222±107 % over basal (P<0.05, paired t-test).

Conclusion: We show that functional responses to 5-HT in human myometrial strips were only observed in the presence of forskolin. The stimulatory effect of agonists may be missed if they are not examined in tissue with different states of activation and relaxation. This finding has profound implications for previous negative studies of stimulatory agonists in vitro.

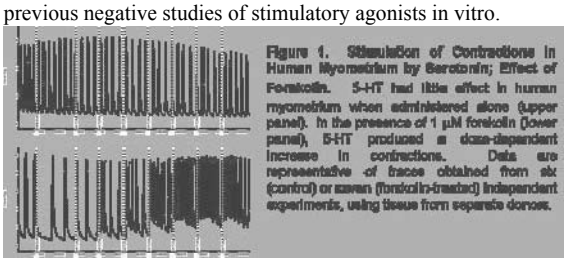


Figure 1. Stimulation of Contractions in Human Myometrium by Serotonin; Effect of Forskolin. 5-HT had little effect in human myometrium when administered alone (upper panel). In the presence of 1 μM forskolin (lower panel), 5-HT produced a dose-dependent increase in contractions. Data are representative of traces obtained from six (control) or seven (forskolin-treated) independent experiments, using tissue from separate donors.

611

Functional and Molecular Characterization of 5-HT_2 Serotonin Receptors in Myometrium from Pregnant Women at Term. Yolande Cordeaux, Hannah Missfelder-Lobos, D Stephen Charnock-Jones,[*] Gordon Smith.[*] *Obstetrics and Gynaecology, University of Cambridge, United Kingdom.*

Introduction: Human myometrium is exposed to 5-hydroxtryptamine (5-HT) from mast cells within the uterus and following release of 5-HT on platelet aggregation. These effects could potentially be modulated by drug treatment and this may be clinically important in preterm labor, abruption and post-partum hemorrhage. However, there has been no systematic study of the pharmacology of 5-HT receptors in human myometrium, to our knowledge.

Methods: Myometrial strips were obtained from women undergoing elective cesarean section (with appropriate consent and ethical approval) and allowed to equilibrate in Kreb's solution for 90mins. A standard contraction was obtained to 50mM KCl and spontaneous contractions were inhibited by 1 μM forskolin. Cumulative concentration response curves (CCRCs) to selective agonists were compared with time and vehicle controls. CCRCs in the presence of selective antagonists were compared with time and vehicle controls. Isometric tension was recorded and analysed using Powerlab® software Chart 5.2.2. Molecular characterization was performed using Western blot analysis and immunohistochemistry

Results: The non-selective 5-HT_2 receptor agonist, α-methyl-5HT, induced a dose dependent stimulation of contractions (pEC_{50} = 7.29±0.19, n=5, maximal contractions; 547±318% over basal) (mean ± s.e). The selective $5HT_{2B}$ agonist BW723C86 and $5HT_{2C}$ agonist Ro 600175 were without significant effect except at concentrations well in excess of their known specificity for these receptors. The $5HT_{2A}$ selective antagonist, ketanserin, caused a parallel shift in the CCRC to α-methyl- 5HT (pK_B= 8.50±0.13, n=6) but had no effect on the response to oxytocin (pEC_{50}s, with and without 1μM ketanserin; 7.45±0.03

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and 7.54±0.03, n=3). The selective $5HT_{2B}$ antagonist SB204741 and $5HT_{2C}$ antagonist RS102221 had no effect on the response to α-methyl- 5HT (pEC_{50}s; 7.38±0.06 (n=3) and 7.23±0.10 (n=4) respectively. pEC_{50} with vehicle: 7.41±0.15, n=4). Western blot using a $5HT_{2A}$ selective antibody confirmed a protein of appropriate molecular weight (56kDa) and immunohistochemisty localized this to myometrial cells.
Conclusions: Myometrium from pregnant women at term expresses functional 5-HT_{2A} receptors. This receptor is a currently unexploited target for novel drug therapy in the clinical control of myometrial contraction.

612

Modulation of Human Myometrial Contractility by Regulators of G Protein Signalling. Claire Hill,[1] Zoe Brownlie,[2] Louise Godfrey,[3] John Davey,[1] Steven Thornton,[*3] Graeme Milligan,[2] Graham Ladds.[3] *[1]Department of Biological Sciences, University of Warwick, Coventry, United Kingdom; [2]Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow, United Kingdom; [3]Division of Clinical Sciences, University of Warwick, Coventry, United Kingdom.*

Objective
G protein-coupled receptors (GPCRs) control all aspects of the contractile process. It follows that molecules which regulate the activity of GPCRs are potential therapeutic targets for controlling pre-term labour. One class of such molecules is the Regulators of G protein Signalling (RGS) which are the focus of our study. Initial analysis has demonstrated RGS2 and RGS5 to predominate within the myometrium [1]. We present data to demonstrate that by a simple single residue mutation we can modulate the activity of these two proteins.
Methods
A previous study isolated an $RGS4^{S30C}$ mutant which displayed enhanced GTPase activity for specific Gα subunits. The residue at position 30 appears to determine the specificity that this enhanced activity demonstrates for the Gα subunits. Analysis of the sequences of RGS2 shows it to contain a homologous serine at position 30 whereas RGS5 contains a proline. Using site-direct mutagenesis we have generated a series of mutations within RGS2/RGS5 and have evaluated their activity within cells.
Results
Continuing from our work with RGS4 we mutated the residues at position 30 within RGS2/RGS5 to include cysteine, phenylalanine and lysine residues. For RGS2 we included a proline mutation. RGS5 however, natively contains a proline at this position which we converted to a serine. All mutant RGS2 and RGS5 constructs were then evaluated for Gα-specific changes in GTPase activity. Manipulation of RGS2 did not affect its ability to modulate Gα signalling, probably due to its ability to function in a GTPase-independent method. Conversion of the proline residue within RGS5 to serine reduces RGS5s activity by approximately 5-fold. Mutations to cysteine, phenyalanine or lysine demonstrate Gα subunit specific enhancements or reductions in signalling.
Conclusions
Mutational analysis of RGS2 and RGS5 has revealed potential differential activities for these RGS proteins within smooth muscle. RGS2 appears to function in a GTPase-independent manner while RGS5 displays varying activities to different Gα subunits. These results should enable a much clear definition of the role these RGS proteins play in the contractile process.
Reference
[1] Zervou *et al., - in press*.

613

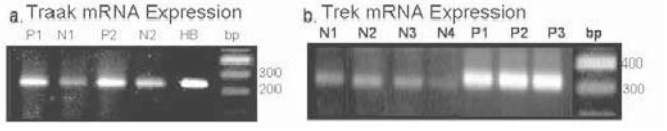
Down Regulation of Prostaglandin $F_{2\alpha}$ Receptor (FP) mRNA by $PGF_{2\alpha}$ and Phorbol Ester Requires Activation of Protein Kinase C in Human Myometrial ULTR Cells. Dean Zaragoza, David M Olson.[*] *Perinatal Research Centre, University of Alberta, Edmonton, AB, Canada.*

Introduction: The action of prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), a potent uterotonic stimulant that is associated with labour at term and preterm, is mediated by its receptor, FP. Myometrial FP mRNA levels fall during pregnancy and rise again at term, but the regulation of these events is poorly understood. We previously found that both $PGF_{2\alpha}$ and the phorbol ester, TPA, which mimics diacylglycerol (DAG) activation of protein kinase C (PKC), surprisingly downregulate FP mRNA expression in cultured ULTR cells. Since binding of $PGF_{2\alpha}$ to its receptor increases DAG levels, we hypothesized that both $PGF_{2\alpha}$ and TPA activate PKC through a classical mechanism that translocates PKC to the cell membrane to mediate its effects.
Methods: Near confluent cultured human myometrial ULTR cells were pretreated +/- the N-terminal myristoylated peptide PKC inhibitor (20-28, 10 μM) for 1h and then treated +/- $PGF_{2\alpha}$(1 μM) or TPA (100 ng/mL) for 6h. Cells were either visualized for localization of conventional PKC isoforms (α,β, and γ) by immunofluorescence, extracted for cytosolic protein and analyzed for PKC activity by ELISA, or extracted for total RNA and analyzed for FP mRNA by real time RT-PCR.
Results: At 6h, $PGF_{2\alpha}$ decreased FP mRNA levels from 1.02±0.06 normalized units in control cells to 0.74±0.03 in treated cells (N=3, $p<0.05$). Pretreatment with 20-28 (10 μM) prevented this downregulation (1.06±0.02 in control/(20-28) treated cells vs. 1.09±0.06 in $PGF_{2\alpha}$/ (20-28) treated cells, N=3, $p=0.68$). There was a tendency for both $PGF_{2\alpha}$ and TPA treatment to decrease calcium and phosphotidylserine dependant cytosolic PKC activity compared to control cells but only the TPA effect was significant (control: 1.96±0.06, $PGF_{2\alpha}$: 1.39±0.24, TPA: 1.28±0.11, N=5-6, $p<0.01$). Importantly, the observed decrease in cytosolic PKC activity coincided with an increase in cell membrane localization for the conventional PKC isoforms.
Conclusions: We conclude that TPA and $PGF_{2\alpha}$ induced down regulation of FP mRNA levels requires activation of PKC through translocation from the cytosol to the cell membrane. These data suggest a paradoxical negative feedback mechanism whereby the increasing levels of $PGF_{2\alpha}$ at labour down regulate expression of FP through DAG activation of the PKC pathway, thereby decreasing myometrial sensitivity to $PGF_{2\alpha}$.
Supported by CIHR.

614

Stretch-Activated Potassium Channels in Human Myometrium. Jennifer N Tichenor, Eric T Hansen, Iain Buxton.[*] *Pharmacology, University of Nevada, Reno, Reno, NV, USA.*

a) OBJECTIVE:
The objective of this research is to determine the role of the stretch-activated potassium (SAK) channels in the human myometrium in order to discover new therapeutic targets for the treatment of preterm labor. Potassium channels that are activated by membrane stretch may contribute to the quiescence of smooth muscle cells in organs that undergo distention, such as the uterus, and have also been found to regulate responses to nitrergic stimulation. We therefore investigated the expression of TREK and TRAAK in human pregnant and non-pregnant myometrium at both an mRNA and protein level.
b) METHODS:
We have employed the methods of semi-quantitative, relative RT-PCR as well as Western Blot in order to determine the mRNA and protein levels of expression of the SAK channels from both pregnant and non-pregnant human myometrial samples.
c) RESULTS:
We show for the first time that TRAAK is present in uterine smooth muscle. We also show that TREK-1 but not TREK-2 is expressed in human myometrium.



Comparison of TREK-1 expression from non-pregnant hysterectomy samples as compared to pregnant cesarean-section samples, each normalized to their respective 18S controls, suggests an up-regulation of TREK-1 channel transcript during pregnancy. Additionally, using Western blot methods, we show that TRAAK protein levels may be up-regulated during pregnancy when comparing pregnant to non-pregnant protein levels.
d) CONCLUSIONS:
The up-regulation of these channels during pregnancy fits with the hypothesis that these channels may be acting to maintain the relative quiescence of the uterine smooth muscle during pregnancy. This mechanism may be improperly regulated in preterm labor, making these as well as functional studies of critical importance to the eventual aim of elucidating a therapeutic target for the prevention and/or treatment of preterm labor.

615

Protein Kinase C and Rho-Associated Kinase Both Regulate Contraction of Pregnant Human Myometrium. Priyan M Tantrige,[1] Andris Kronbergs,[2] Victor P Fomin.*[2] [1]*Imperial College, London, United Kingdom;* [2]*Biological Sciences, University of Delaware, Newark, DE, USA.*

Ca sensitization is a physiological phenomenon during which smooth muscle produces more contraction at given level of intracellular Ca. While the effect is well characterized in vascular smooth muscles, its contribution to uterine contraction is less certain. Rho-associated kinase(ROK) and protein kinase C (PKC) were shown to mediate Ca-sensitizing effect of agonists in a number of is smooth muscles. **Objective:**This study was designed to elucidate the effect of PKC and ROK on $[Ca^{2+}]_I$, force and their relationship in myometrium from term pregnant women. **Methods:** The isometric force and $[Ca^{2+}]_I$ were measured simultaneously in fura-2 loaded myometrial strips using spectrofluorometer equipped with force transducer. The uterine contractile responses were recorded with force transducer using organ bath. **Results:** The PKC activator phorbol 12,13-dibutyrate (PDBu) produced sustained uterine contraction in time and dose dependent manner with a maximal effect at 10^{-6} M PDBu after 2hrs incubation. At the same time, no significant effect of PDBu on basal level of $[Ca^{2+}]_I$ was observed. The effect was also seen in Ca-free media and when intracellular Ca was "clamped" with BAPTA. PKC inhibitor GF109203X and ROK inhibitor Y27632 both added at the peak of 10^{-7}M oxytocin-induced contraction reduced the contraction without affecting the $[Ca^{2+}]_I$ level. Addition of one inhibitor after the other one (Y27632 after GF109203X or visa versa) didn't result in further decrease of the contraction. Likewise these inhibitors added at 10^{-5}M at the plateau of the 50mM KCl-induced contraction had similar effect on the contraction with no effect on the $[Ca^{2+}]_I$. Again the subsequent addition of one inhibitor over the other didn't yield in additional decrease of the contraction. Relatively specific PKCα inhibitor Go6976 and PKCβ specific inhibitor both at 10^{-5}M, added at the peak of KCl-induced contraction produced 15-20% inhibition of the contraction. **Conclusion:** We suggest that both PKC (possibly α and β isoforms) and ROK can regulate uterine contraction through Ca-sensitizing mechanism. Since the effect wasn't additive, it is likely that PKC and ROK affect the same down-stream mechanism (possibly myosin light chain phosphatase) to regulate uterine contractile responses to various stimuli (Supported by NIH R55 HD45802).

616

Two Components in the Oxytocin-Induced Potentiation of Myometrial Contractility. Joanna E Gullam, Andrew M Blanks, Steven Thornton,* Anatoly Shmygol. *Warwick Medical School, The University of Warwick, Coventry, United Kingdom.*

Oxytocin-induced release of Ca^{2+} ions from sarcoplasmic reticulum (SR) and sensitisation of contractile proteins to Ca^{2+} mediate the oxytocin-induced potentiation of myometrial contractions. **Objective:** We investigated the mechanism of action of oxytocin using pharmacological tools to manipulate the excitation contraction coupling. **Method:** Samples were obtained from women undergoing Caesarean Section with the approval from the Local Ethics Committee. Strips were mounted in an organ bath (AD Instruments, UK) and stable spontaneous contractions were recorded for 40-60 min before addition of oxytocin. Myometrial contractility was measured as 30 min time integral of contractions before and after addition of drugs. **Results:** Prolonged (60-120 min) application of oxytocin (0.1 – 100 nM) produced a two-component effect. At the beginning of oxytocin application, there was an early component, a tetanus-like contraction which lasted 5-15 min. This was followed by a late component of long-lasting augmentation of phasic contractions. A phospholipase C inhibitor U73122 (10 - 30 uM) substantially decreased spontaneous contractions, partially inhibited the early component but failed to abolish the late component of the oxytocin effect. Disabling the SR Ca store using thapsigargin abolished the initial component but did not change the late component. An IP_3 receptor and store-operated Ca channels inhibitor, 2-aminoethyldiphenyl borate (2-APB) significantly decreased spontaneous contractions and both components of the oxytocin-induced potentiation of contractions. A Rho-kinase inhibitor Y27632 decrease spontaneous contractions, had no effect on the early component and substantially, although not completely, inhibited the second component of the oxytocin effect. **Conclusions:** Based on these results, we propose that the early component of the oxytocin effect is due to the IP_3–mediated Ca^{2+} release from the SR. This early component wears off as soon as the released $Ca^{2+}$is removed from the cytoplasm. The late component of the oxytocin effect manifests itself as potentiated phasic contractions and lasts as long as oxytocin is present. Rho-kinase is involved in oxytocin-induced potentiation of phasic contractions, but is not the only mechanism responsible. Since 2-APB affected both components, the store activated Ca channels might be involved in the late component of oxytocin-induced potentiation of myometrial contractility.

617

Localisation and Functional Characterisation of Bradykinin Receptors in Human Myometrial Arteries. Amie J Bowler,[1,2] Gillian A Gray,[1] David E Newby,[1] Fiona C Denison.[2] (SPON: Hilary OD Critchley). [1]*Centre for Cardiovascular Science, The Queens Medical Research Institute, Edinburgh, United Kingdom;* [2]*Centre for Reproductive Biology, The Queens Medical Research Institute, Edinburgh, United Kingdom.*

Objective: To characterise the response to bradykinin (BK) using selective B_1 and B_2 receptor agonists and antagonists in isolated myometrial arteries from healthy pregnant women.

Methods: Arteries (200-400μm) dissected from myometrial biopsies were mounted on a wire myograph. Dose response curves to BK, a B_2 receptor agonist and Lys-des-Arg^9-bradykinin (LDABK), a B_1 receptor agonist (10^{-11}–10^{-6}M) were constructed in the presence and absence of selective B_1 (des-arg^{10}-HOE 140) and B_2 (HOE 140) receptor antagonists (10^{-6}M) (n=6). Experiments were repeated in the presence of cyclohexamide (70μM) to investigate post-isolation induction of the receptors (n=9). Umbilical vein studies were used to confirm the efficacy of the drugs used. B_1 and B_2 receptors were immunolocalised using goat polyclonal antibodies.

Results: Arteries pre-constricted with the thromboxane mimetic U46619 exhibited concentration-dependent relaxation to BK (EC_{50}=3.44x10^{-9}M). The B_1 receptor antagonist had no effect on BK-induced relaxation however the B_2 receptor antagonist significantly inhibited the effects of BK (p= <0.0001). The B_1 receptor agonist LDABK produced a biphasic response with vasorelaxation at low doses (10^{-11}-10^{-8}M) and vasoconstriction at higher doses (10^{-8}-10^{-6}M). The response observed using LDABK was not inhibited by either B_1 or B_2 receptor antagonists. Both antagonists were efficacious in the umbilical vein. Cyclohexamide blocked the effect of LDABK in some vessels but not in others and had no effect on BK B_2-induced relaxation. Immunohistochemistry studies demonstrated strong immunolocalisation of the B_2 but not B_1 receptor to the endothelium of vessels, including those used for myography that responded to LDABK. In addition, B_1 receptor immunolocalisation was observed in areas where trophoblast invasion had occurred. This was confirmed by positive staining for cytokeratin.

Summary: BK-mediated relaxation of myometrial arteries is via activation of B_2 receptors on endothelial cells. However, an additional pathway is present that can be activated by the B_1 receptor agonist LDABK. This involves a non B_1/B_2 receptor mechanism that can be up-regulated *in vitro*. Association of B_1 immunoreactivity with trophoblasts may suggest an additional role of BK in mediating trophoblast invasion of the myometrium.

618

Activation of at Least Three Classes of Ion Channels by β-Adrenoceptor Activation in Pregnant Uterine Smooth Muscle. Helena C Parkington, Mary A Tonta, Sonya Simon, Saul A Cohen, Alex Satragno, Wee-Ming Boon, Marianne Tare, Harold A Coleman, Richard J Lang. (SPON: Elvie M Wintour). *Physiology, Monash University, Melbourne, Victoria, Australia.*

Preterm labour is associated with significant adverse neonatal outcomes, and may have lifelong consequences for health. Efforts to suppress uterine contractions are hampered by incomplete understanding of mechanisms involved. Ca^{2+} influx and membrane potential (MP) are critical determinants of contraction. We have previously shown that activation of β-adrenoceptors in sheep myometrium induces large hyperpolarization that is inhibited by blockade of ATP-sensitive K^+ (K_{ATP}) channels. We investigated the mechanisms further studying: 1) channel function in isolated myocytes, 2) tension, Ca^{2+} and MP in myometrial strips, and 3) uterine electrical and contractile activities in ewes in labour.

Hyperpolarizations (15 mV, n=20) evoked in myometrial strips by salbutamol (SAL, 10-300 nM) were blocked by glibenclamide (GLI, 1 μM, n=16) and PNU-37883A (10 μM) but not by iberiotoxin (IbTx, 60 nM). SAL prominently activated BK_{Ca} channels in myocytes and caused a leftward shift of the activation curve that was similar to raising intracellular $[Ca^{2+}]$ (n=12). Similar results were obtained in term (not in labour) human myometrium. Western blot analysis revealed KIR6.1, KIR6.2, SUR2A, SUR2B and BK_{Ca} protein in human and sheep myometrium. Blockade of BK_{Ca} channels with IbTx revealed that SAL also activated two channels, a K_{ATP} channel of 62pS and a channel (14 pS) that reversed near -20mV. In strips in the presence of GLI, action potential amplitude was reduced by SAL, and this was blocked by IbTx. In the presence of PNU-37883A, SAL induced a small depolarization.

SAL infusion (100 μg/kg/hr) into conscious ewes caused cessation of bursts of EMG activity and contractions. Following 30 min infusion of GLI (1mg/kg/hr), SAL failed to suppress uterine activity before (n=7) and during normal spontaneous labour (n=3), and following induction of labour preterm (n=5).

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These results demonstrate a significant activation of K_{ATP} by SAL in pregnant human and sheep myometrium at the single channel and tissue levels, and in the intact ewe. BK_{Ca} channels are also activated, but their main effect is not to cause membrane hyperpolarization. An intriguing action of SAL was the activation of an inward conductance, but its role in the excessive "rebound" uterine activity seen following SAL withdrawal *in vivo* awaits further investigation.

619

Endothelial and Myometrial Cell Changes in the Human Uterus at Term. Roger Smith,[*1] Anthony Leong,[2] Jane E Norman.[*3] [1]*Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia;* [2]*Anatomical Pathology, University of Newcastle, Newcastle, NSW, Australia;* [3]*Obstetrics and Gynaecology, University of Glasgow, Glasgow, Scotland, United Kingdom.*

This study examined the histology of human term myometrial samples to identify morphological changes that may elucidate the mechanisms that initiate inflammatory processes at the time of labor.

Methods: Lower uterine segment myometrial biopsies obtained from 53 patients at term not in labour and 26 patients in labour. All cases were stained with an anti-macrophage antibody (CD68), anti-CD34 to endothelial cells and anti-Caspase 3 to label apoptotic cells (1:200), using a strepavidin-peroxidase technique following microwave antigen retrieval. All sections were observed for the following: inflammatory response, particularly of neutrophilic infiltration; macrophage infiltration based on immunostaining with anti-CD68 and vascularity of the section based on CD34 immunoexpression by endothelial cells. The neutrophilic infiltration was scored on a semi-quantitative basis where 0 = no or infrequent neutrophils; 1+ = scattered or 0-5 neutrophils present in a perivascular location; 2+ = moderate or > 5 neutrophils present in a perivascular location; 3+ = neutrophils present in the interstitium and myometrium. The infiltration of macrophages was scored in an identical manner using CD68 as a marker. Apoptotic cells were assessed by nuclear staining of Caspase 3.

Results: The samples showed a variable inflammatory response of neutrophils and macrophages, mostly localised to perivascular locations with a greater infiltrate in the samples obtained during labour. In addition, there were prominent changes in the myometrial fibres including shearing, shrinkage, oedema and particularly apoptosis even in samples obtained from women clinically not in labour. Unexpectedly endothelial cells of thin walled vessels prominent in the biopsies displayed marked biotinylation and the vascular lumen contained fibrin and platelet thrombi as well as desquamated endothelial cells and amniotic squamous cells and mucoid material.

Conclusions: These findings suggest that endothelial cell damage and amniotic fluid embolism are very common at term prior to clinical labour and provide a mechanism by which surfactant protein A and phospholipids present in the amniotic fluid may access myometrial cells and provoke the inflammatory response that occurs during parturition.

620

Expression of NFAT Transcription Factor Isoforms in Human Myometrium. Frances R Willey,[1] Verity Kew,[1] Emily Oliver,[1] Suren R Sooranna,[2] Laura A McCallum,[1] Rachel M Tribe.[*1] [1]*Division of Reproduction and Endocrinology, King's College London, London, United Kingdom;* [2]*Department of Maternal and Fetal Medicine, Imperial College School of Medicine, London, United Kingdom.*

Objective: The family of nuclear factor of activated T-cells (NFAT 1-4) isoforms are transcription factors that play a central role in mediating calcium-sensitive gene activation. In most resting cells, NFATs are phosphorylated at a cluster of serine residues located in the regulatory domain, covering a nuclear localization signal, thereby retaining NFAT in an inactive conformation in the cytoplasm. Dephosphorylation of NFATs, which allows relocation to the nucleus, is associated with a rise in intracellular calcium and the activation of calcineurin (a phosphatase). In the nucleus NFATs then bind to the promoter regions of target genes, including TrpC1, a putative calcium channel present in human myometrium. Despite the important role NFAT isoforms play in other cell types, little is known about NFAT expression and regulation in human myometrium. The aim of this study was to determine which NFAT isoforms are expressed in human myometrial tissue and cells.

Methods: Lower uterine segment myometrial biopsies were obtained with informed written consent from women undergoing elective caesarean at term. Tissues were either snap frozen in liquid nitrogen or primary uterine myocytes were prepared and cultured in medium containing 5% fetal calf serum. NFAT 1-4 gene expression was assessed in tissue and primary cultured cells by RT-PCR (n = 4-8) and NFAT 3 and NFAT 4 protein expression was measured in cultured cells by Western blot (n = 6).

Results: NFAT 3 and NFAT 4 mRNA, but not NFAT 1 and NFAT 2 were detected in human myometrial tissue and primary cells (n = 4-8). NFAT 3 and NFAT 4 protein expression was confirmed in cultured cells (n = 6).

Conclusions: These results indicate a possible role of NFAT 3 and NFAT 4 in the regulation of gene expression of human myometrium of term and suggest that further investigation of NFAT isoforms in human myometrium is warranted.

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621

Expression of KCNQ and KCNE Genes in Non-Pregnant Mouse Myometrium and in Non- Labouring Human Myometrial Tissue. Laura A McCallum,[1] Iain A Greenwood,[2] Frances Willey,[1] Rachel M Tribe.[*1] [1]*Division of Reproduction and Endocrinology, King's College London, London, Greater London, United Kingdom;* [2]*Division of Basic Medical Sciences, St George's University of London, London, Greater London, United Kingdom.*

Background: The KCNQ (Kv7) family of potassium channels encode a family of K^+ channel α-subunits which have been most extensively investigated in cardiomyocytes and the CNS. Little is known about the contribution of KCNQ channels to uterine smooth muscle function, but expression has been noted in both mouse portal vein and rat stomach smooth muscle (Yeung & Greenwood 2005, Ohaya *et al.* 2003). KCNQ proteins can form homomultimers and/or heteromultimers with other members of the family and are regulated by subunits encoded by KCNE genes. The aim of this study was to examine the expression of the 5 KCNQ genes and the 5 KCNE genes i) during the oestrous cycle in mice and ii) in term non-labour (TNL) human myometrial biopsies.

Methods: Oestrous cycles were monitored in C57/BL6 mice by daily vaginal smearing and uterine horns dissected at the appropriate time: diestrous (n=5), proestrous (n=5), oestrous (n=6), metestrous (n=6). Human myometrial tissue was obtained with informed consent from women undergoing cesarean section (38-41 weeks) (n=5-6). Total RNA was extracted using Trizol (Invitrogen) and cDNA synthesised for use in (real-time) RT-PCR using Superscript III (Invitrogen). Real-time RT-PCR data was normalised to β-Actin.

Results: KCNQ1-5 and KCNE1-5 were detected in mouse myometrium at all stages of the oestrous cycle using RT-PCR. Real-time RT-PCR indicated that KCNQ5 and KCNE3 were the most abundantly expressed genes. KCNQ5 was 429 times more abundant than KCNQ1 (95% CI, 375-4951, $p < 0.00001$) and KCNE3 was significantly higher than KCNE2 ($p < 0.05$). KCNE3 was significantly down regulated in metestrous ($p = 0.01$) whereas KCNE4 was significantly upregulated in proesterous ($p = 0.01$). KCNQ1, KCNQ2, KCNE1a/b, KCNE2, KCNE3 and KCNE4 mRNA was detected in human myometrium by RT-PCR.

Conclusions: The detection of KCNQ and KCNE isoforms in human and mouse myometrium suggests they may play a role in the control of uterine function. The preferential variability in the KCNE subunits KCNE3 and KCNE4, rather than in KCNQ mRNA, suggests that hormonal regulation of channel function is at the level of the regulatory subunit.

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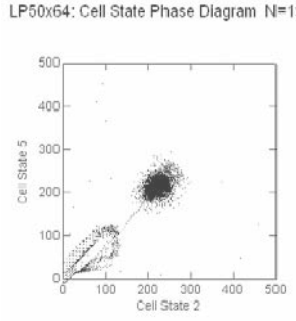
622

Emergent Phenomena in Cellular Automata Designed To Model the Human Uterus in Labor. Mel L Barclay,[1] HF Andersen,[2] Carl P Simon.[3] (SPON: Timothy RB Johnson). [1]*Department of Obstetrics and Gynecology, University of Michigan School of Medicine, Ann Arbor, MI, USA;* [2]*Maternal Fetal Medicine, Providence Everett Medical Center, Everett, WA, USA;* [3]*Department of Mathematics, University of Michigan, Ann Arbor, MI, USA.*

Objective: To utilize a computer model of the human uterus constructed of discrete cellular elements to explore the impact of geometric alterations in shape and pacemaking activity on contractile patterns and the appearance of emergent forms of activity, which resemble variations in normal and abnormal labor.

Methods: Two methods were utilized to model the anatomy of human uteri. In the first, ellipsoids of revolution were produced and in the second, rings of cells were utilized to build constructions which resemble the structure of the normal human uterus. Varied numbers of cells from 847 to 10,222 were utilized in the experiments. Each cell is connected to its neighbors electrically according to the Turing scheme. Cells are characterized as excitable tissues

which have the property of stimulation, propagation, a refractory phase, and pressure production according to the Hodgkin-Huxley relationships and Laplace's Law.
Results: Many complex patterns of behavior arise when small changes are made in the anatomy, activation, and location of pacemakers. Vortices and fractal basins of attraction appear (see fig) which are characteristic of many systems composed of excitable tissues. Patterns of depolarization emerge which sometimes result in regular, periodic, or quasiperiodic contraction cycles, and which mimic patterns seen in electrohysterographic experiments in humans. In some models, organization develops which is complex and stable, yet does not produce regular waves of contraction pressure or patterns which could be associated with normal labor and orderly cervical dilatation. Other models quickly develop patterns of contractile behavior which, in humans, would be associated with progressive dilatation of the cervix and productive labor. Small adjustments in the numbers or locations of resting cells have dramatic impact on the overall characteristics of the modelled behavior.



623

A New Model To Study Myometrial Complexity and Heterogeneity. Gilles Bru-Mercier, Anatoly Shmygol, Steven Thornton,* Andrew M Blanks. *Warwick Medical School, University of Warwick, Coventry, United Kingdom.*
Introduction: It is well established by previous works that intercellular communications, through gap-junctions and through paracrine interactions, are important for synchronizing the activity of uterine myocytes. Cultured myometrial cells, often used in current research, are a convenient but not ideal model to study intercellular communications in human myometrium. **Objective:** To investigate the mechanisms of communication between uterine myocytes in their natural environment. **Methods**: Myometrial biopsies were taken from patients undergoing Caesarean Section with informed written consent (REC-05/Q2802/107). The slices, 200 µm thick, were cut using a vibroslicer in Krebs solution (4°C) and cultured in SmGM-2 (Cambrex) medium, 5% FCS. Experiments were performed on slices on each day of a total of 9 days in culture. Each slice was loaded with Ca^{2+}-sensitive Fluo-4 and imaged at 1 frame/s on a Zeiss LSM 510 META confocal. To study the evolution of the intercellular communication during the culture, we recorded spontaneous activity at 37°C and responses to 100 nM oxytocin (OT). **Results:** All samples tested (n=11) demonstrated spontaneous contractile activity on the day of isolation. During spontaneous and OT induced contractions increases in fluorescence were indicative of action potentials (AP) with a fast spike followed by sustained plateau. This AP-like response was lost in culture in association with loss of synchrony. Over time in culture there was a marked decrease in synchrony. Interestingly, agonist mediated responses demonstrated initial calcium transients origination from the interstitial spaces followed by cohesive AP's in myometrial smooth muscle cell (MSMC) bundles. During relaxation desynchronised sub-cellular calcium oscillations were often observed in MSMC bundles. After time in culture and loss of synchrony, some cells demonstrated spontaneous calcium oscillations that were unaffected by agonist.**Conclusion**: The ultra-thin slices of human myometrium are a powerful experimental model to study myometrial intercellular communications. Initial work demonstrates a previously unrecognized level of complexity in calcium signalling in human myometrium incorporating physical structure, spatio-temporal phenomena, intercellular connections and cellular heterogeneity. This approach provides large amounts of information for the study of different components of myometrial contractions, communication and signalling.

624

Inhibition of Gene Expression Using RNA Interference Technology in Human Myometrial Tissue Strips. Kristina M Fetalvero,[1,3] PeiSheng Zhang,[2] Jin Zhang,[2] Robert J Wagner,[3] Kathleen A Martin,[1,3] Roger C Young.[*2,4] *[1]Pharmacology, Dartmouth-Hitchcock Medical Center, Hanover, NH, USA; [2]Obstetrics and Gynecology, Dartmouth-Hitchcock Medical Center, Hanover, NH, USA; [3]Surgery, Dartmouth-Hitchcock Medical Center, Hanover, NH, USA; [4]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*
Objective: RNA interference technology has proven broadly useful in obtaining detailed understanding of specific gene action on cellular function. We have previously published a method for culturing tissue strips of human myometrium in organ culture such that contractile responses are expressed > 7 days. This work aims to join these techniques to allow extension of siRNA technology to human myometrial contractility at the tissue-level.
Methods: Following informed consent, myometrial tissue was obtained from the lower uterine segment of term pregnant women at the time of cesarean delivery. Tissue strips were cultured under tension (400 mg weight) in 5 mL DMEM with 10% FBS. After 24 hours, siRNA (2 nM final concentration) was added using Dharmafect 3 reagent (Dharmacon). Tissue was lysed 48 hours later and equal microgram amounts of protein per sample were analyzed by western blotting with antibodies to GATA-6, beta tubulin, and smooth muscle myosin heavy chain (SM-MHC).
Results: We compared expression of the smooth muscle transcription factor GATA-6 after siRNA transfection of GATA-6 siRNA or a scrambled, non-targeting siRNA. Samples were normalized for protein loading to levels of the housekeeping gene beta tubulin. Compared to the negative control siRNA, expression of GATA-6 protein was reduced by 50% after transfection with the GATA-6 siRNA, and a 50% knockdown in expression of the GATA-6 transcriptional target, SM-MHC, was found.
Conclusions: Here we demonstrate the ability to knock down expression of a target gene by 50% in human myometrial organ culture. This technology will likely allow systematic assessment of other genes with regard to mechanisms of myometrial contractility, thereby enhancing our understanding of the molecular mechanisms underlying human labor. As GATA-6 is known to be an important regulator of smooth muscle-specific contractile protein genes in human vascular smooth muscle, this technique clearly demonstrates a role for GATA-6 in regulating contractile proteins in uterine smooth muscle.

625

Differential Expression of 20α-Hydroxysteroid Dehydrogenase (*AKR1C1*) but Not 5α-Reductase, Type 1 (*SRD5A1*), Occurs with Human Labor Induction. Richard H Lee,[1] Frank Z Stanczyk,[*1] Qing Ji,[2] Andrew Stolz,[2] Wangrong Yang,[1] Thomas M Goodwin.[*1] *[1]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA; [2]Gastrointestinal and Liver Disease, University of Southern California, Los Angeles, CA, USA.*
OBJECTIVE: To determine the relative expression of 20α-hydroxysteroid dehydrogenase (*AKR1C1*) and 5α-reductase type 1 (*SRD5A1*) in the myometrium and chorioamniotic membranes in three states of human labor: not in labor, spontaneous labor, induced labor.
METHODS: Quantitative real-time RT-PCR was used to compare relative expression of 20α-hydroxysteroid dehydrogenase (*AKR1C1*) and 5α-reductase type 1 (*SRD5A1*) in two tissues (myometrium and chorioamniotic membranes) in 23 subjects in three different states of labor: not in labor (n=13), spontaneous labor (n=5), induced labor (n=5). Labor was defined as regular uterine contractions that resulted in cervical dilation. Induced labor was defined as the need for an induction agent to begin the labor process (pitocin=1, foley=2, dinoprostone insert=2).
RESULTS: Expression of 20α-hydroxysteroid dehydrogenase was significantly higher in the myometrium of spontaneous laboring subjects compared to those not in labor (2.5fold, P=0.034) and induced labor (3.25 fold, P=0.028). Regardless of labor status, there was no difference in 20α-hydroxysteroid dehydrogenase expression between the groups in the chorioamniotic membranes. Expression of 5α-reductase type 1 was significantly lower in the membranes of both laboring groups when compared to not in labor (spontaneous[0.14 fold, P=0.027]; induced [0.11 fold, P=0.02]). Regardless of labor status, there was no difference in 5α-reductase type 1 expression in the myometrium.
CONCLUSIONS: Our finding of increased expression of 20α-hydroxysteroid dehydrogenase in myometrium is consistent with a process of local progesterone withdrawal associated with spontaneous labor, but not induced labor. Decreased expression of 5α-reductase in membranes of women in labor whether spontaneous or induced suggests that local withdrawal of the metabolically active metabolite 5α-dihydropogesterone is a characteristic of the labor process in general.

626

The Prudent Maternal Dietary Pattern Reduces the Risk of Having a Child with Spina Bifida. Marijana Vujkovic,[1] Marga C Ocke,[2] Peter J van der Spek,[3] Eric AP Steegers,[*1] Regine PM Steegers-Theunissen.[1] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]National Institute for Public Health and the Environment, Bilthoven, Netherlands; [3]Bioinformatics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

BACKGROUND: Periconceptional nutrition of the mother-to-be is associated with the occurrence of spina bifida offspring (SB). Sofar, studies focussed on the identification of single nutrients in the maternal diet only.

OBJECTIVE: To identify maternal dietary patterns in association with the risk of having a child with SB.

STUDY DESIGN: From 106 mothers of a child with SB and 166 control mothers dietary patterns were computed from food frequency questionnaires (FFQ) collected at 14 months after the index-pregnancy as a proxy of the periconceptional dietary intake. Maternal blood samples were obtained for the determination of the concentrations of folate, vitamin B12, pyridoxal '5' phosphate, total homocysteine (tHcy), myo-inositol and glucose. Odds ratios and 95% confidence interval (OR, 95%CI) for each tertile of the dietary pattern were calculated to estimate SB risk.

RESULTS: We identified 3 major dietary patterns, the Prudent, Mediterranean and Snack dietary pattern (Figure). Mothers with a high Prudent score are in general older, higher educated and smoked less. Users of the Mediterranean diet with a high score are older, higher educated, consumed more alcohol and had a lower total energy intake. The Snack pattern with a high energy intake is used by younger and lower educated mothers. The Prudent diet decreased SB risk by 50% (OR= 0.5, 95%CI, 0.3 - 0.95), whereas the Snack pattern increased SB risk nearly 2-fold, OR 1.8 (95%CI 1.0 - 3.2). Significant are the associations between the Snack pattern and a high tHcy (p-trend= 0.009) and a low vitamin B12 concentration (p-trend= 0.027).

CONCLUSION: Mothers on a Prudent or Mediterranean diet use a healthier lifestyle. This is in contrast to the users of a Snack pattern. In preconception counseling the use of the Prudent diet should be recommended and the Snack pattern should be stopped.

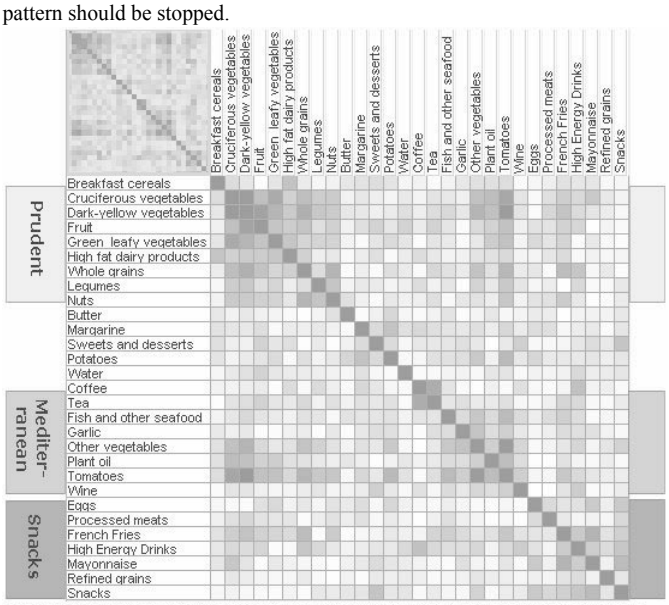


Figure: A Pearson correlation plot displays all the individual correlation coefficients between the food groups. The food groups are clustered based on the strongest relationships. Each cluster of food groups defines a dietary pattern.

627

Low Maternal Dietary Intake of Docosahexanoic Acid, Riboflavin and Niacin Leads to Congenital Heart Defects in Human. Huberdina PM Smedts,[1] Maryam Rakhshandehroo,[1] Anna C Verkleij-Hagoort,[1] Jeanne H de Vries,[2] Eric AP Steegers,[*1] Regine PM Steegers-Theunissen.[1,3,4,5] *[1]Obstetrics and Gynecology/Div of Obstetrics and Prenatal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Human Nutrition and Epidemiology, Wageningen University, Wageningen, Netherlands; [3]Epidemiology and Biostatistics; [4]Pediatric Cardiology; [5]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

BACKGROUND: Besides the role of folic acid, less evidence is available on other nutrients in the pathogenesis and prevention of Congenital Heart Defects (CHD). The B vitamins riboflavin and niacin serve as cofactors in the synthesis and degradation of fats. These nutrients are implicated in molecular biologic processes involved in the embryogenesis of the heart.

OBJECTIVE: To investigate associations between the maternal dietary intake of fats, riboflavin and niacin and the risk of CHD offspring.

METHODS: A case-control family study of 192 mothers of a child with CHD and 216 mothers of a healthy child was conducted in the Netherlands. A general and a food frequency questionnaire, covering the intake of the previous month, were filled out at 17 months after the index-pregnancy as a proxy of the periconceptional dietary intake. Mothers who were pregnant, lactating, or those who reported an altered diet compared to the periconceptional period were excluded for analysis. Univariate logistic regression analyses were performed.

RESULTS: The intake of total fat, saturated and mono- and polyunsaturated fatty acids was not significantly different between case- and control mothers. A significant trend (p-value 0.02) towards a nearly 3-fold increased risk of CHD was demonstrated for a low (<0.02 g/day) intake of the N-3 fatty acid docosahexanoic acid (DHA) by mothers not using a vitamin supplement in the periconceptional period, OR 2.9(95% CI 1.03-7.9). Case-mothers showed a significantly lower intake of niacin than controls ($p \leq 0.05$). Moreover, a low intake of riboflavin (<1.1 mg/day) was associated with a more than 3-fold increased CHD risk in non-supplement users, OR 3.5 (95%CI 1.1-10.8).

CONCLUSIONS: Our findings suggest that in addition to folic acid, a maternal diet rich in riboflavin, niacin and DHA may contribute to a reduction in CHD risk. This should, however, be confirmed in a randomised controlled trial.

628

Periconceptional Multivitamin Use Is Associated with Reduced Risk of Preterm or Small for Gestational Age Births. Janet M Catov,[1,3] Lisa M Bodnar,[1,2,3] Roberta B Ness,[1,2,3] James Roberts.[*1,2,3] *[1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Ob/Gyn & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [3]Magee Womens Research Institute, Pittsburgh, PA, USA.*

Objective: Evidence relating periconceptional vitamin use to preterm or small for gestational age (SGA) births is conflicting. The objective was to determine the relation between periconceptional multivitamin use and the risk of SGA births (<5th percentile and 5th-<10th percentile) or preterm births (<34 weeks and 34-<37 weeks).

Methods: Pregnant women without chronic conditions or preeclampsia (n=1746) in the Pregnancy Exposures and Preeclampsia Prevention Study (Pittsburgh PA 1997-2001) reported whether they had used multivitamins regularly or not in the 6 months before study enrollment (mean 10 weeks, SD 4). Women were classified as users (n=817) and non-users (n=929) in multinomial logistic models.

Results: After adjusting for race, education, gestational age at enrollment, and household density, periconceptional multivitamin use was associated with a reduced risk of preterm birth <34 weeks of 60% (OR 0.40; 95% CI 0.17-0.96). Risk of SGA <5th percentile was 42% lower (OR 0.58; 95% CI 0.35-0.97) after adjustment for smoking, education, parity, gestational age at intake, and BMI. Results were unaltered after adjustment for additional socioeconomic, lifestyle and pregnancy characteristics. Sensitivity analysis for unmeasured confounding by folate intake supported these findings. There was no relation between periconceptional multivitamin use and moderate preterm birth (34-<37 weeks) or moderate SGA (5-<10th percentile). Pre-pregnancy BMI modified the relationship between periconceptional multivitamin use and risk of preterm or SGA births. Non-obese women who used multivitamins had a 69% (OR 0.31; 95% CI 0.11-0.86) reduction in preterm birth risk <34 weeks, and a 47% (OR 0.53; 0.3-0.9) reduction in risk of SGA <5th percentile compared with non-obese non-users. There was no association among obese women.

Conclusion: Our results indicate lower rates of preterm birth and SGA in lean women who report vitamin use before pregnancy. This suggests that multivitamin use in the periconceptional period could be an inexpensive and acceptable intervention to reduce risk of SGA and early preterm birth, especially among non-obese women.

629

Maternal Riboflavin Intake and Two MTHFR Polymorphisms and the Association with Cleft Lip and/or Palate Offspring. Marijana Vujkovic,[1] Joyce van Meurs,[2] Nahid Yazdanpanah,[2] Andre G Uitterlinden,[2,3] Eric AP Steegers,[*1] Regine PM Steegers-Theunissen.[1,3,4,5] *[1]Obstetrics and Gynecology; [2]Internal Medicine; [3]Epidemiology and Biostatistics; [4]Pediatric Cardiology; [5]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

BACKGROUND: Riboflavin is a cofactor of the MTHFR enzyme involved in homocysteine remethylation. An inverse relationship exists between riboflavin and homocysteine concentrations. Maternal hyperhomocysteinemia is a risk factor for cleft lip and/or palate (CLP) offspring.

OBJECTIVE: To investigate associations between maternal dietary riboflavin intake, use of folic acid or multivitamin supplement, MTHFR C677T and A1298C genotypes, and CLP risk.

Methods: A case-control family study was conducted in 141 CLP and 127 control mothers and children. Mothers filled out a general and a food frequency questionnaire at 14 months after the index-pregnancy as a proxy of the periconceptional diet. MTHFR C677T and A1298C genotypes were determined in mothers and children. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated.

RESULTS: We did not find a significant influence of dietary riboflavin intake on the association between the MTHFR C677T and A1298C genotype and CLP risk. However, mothers with MTHFR 1298AC or CC genotypes, who had a low riboflavin intake and did not use a supplement in the periconceptional period demonstrated a 7-fold increased risk of CLP offspring (Table).

CONCLUSION: Periconceptional riboflavin supplementation may contribute to the prevention of CLP offspring in particular in nonsupplemented mothers with a low riboflavin intake and carrying MTHFR 1298AC or CC genotypes.

Maternal MTHFR A1298C genotypes, periconceptional dietary riboflavin and vitamin supplement intake, and CLP risk

supplement1	MTHFR A1298C	riboflavin intake	CLP, n (%)	Controls,n(%)	OR,95%CI
No	AC/CC	Low	11 (28.2)	2 (7.1)	7.0, 1.3-36.3
		High	28 (71.8)	26 (92.9)	1.4, 0.6-3.2
	AA	Low	10 (37)	7 (28)	1.8, 0.6-5.9
		High	17 (63)	18 (72)	1.2, 0.5-3.1
Yes	AC/CC	Low	2 (16.7)	5 (21.7)	0.5, 0.1-3.0
		High	10 (83.3)	18 (78.3)	0.7, 0.3-2.0
	AA	Low	3 (16.7)	6 (24)	0.6, 0.1-3.0
		High	15 (83.3)	19 (76)	Reference

[1]multivitamin and/or folic acid supplement. MTHFR=methylenetetrahydrofolate reductase,OR, 95% CI=odds ratio with 95% confidence interval

630

Maternal Serum and Fetal Cord Leptin Levels at Term: Correlations with Race and Weight. Christine W Mansfield, Angela G Duke, Barbara A Staton, C Richard Parker.[*] *Department of OB GYN, University of Alabama at Birmingham, Birmingham, AL, USA.*

Objective:

To determine if racial differences in maternal and fetal cord blood leptin concentrations exist between black and white women at the time of cesarean section.

Methods:

Specimens were collected from elective cesarean sections and stored frozen at -20 degrees C prior to assay for leptin by RIA. Maternal venous samples were collected at admission and umbilical vein cord blood samples were collected immediately after cord clamping.

Results:

We obtained maternal serum from 32 black women and 18 white women. We were also able to obtain umbilical vein serum in 17 black participants and 9 white participants. Maternal weight averaged 88 kilograms in black women and 78 kilograms in white women, but this difference was not statistically significant. Infant birth weights were very similar, averaging 3261 grams in black infants and 3246 grams in white infants. The maternal leptin levels in black women (22.2 +/- 2.4ng/ml, Mean +/- SE) were not statistically significantly different from white women (18.4 +/- 2.0ng/ml). Infant cord leptin levels in black infants (9.91 +/- 2.4ng/ml) were not statistically significantly different than white infants (11.82 +/- 3.8ng/ml). The leptin concentrations in maternal and umbilical venous cord samples were within the range reported by other researchers. There was a strong positive correlation between leptin levels and maternal weight in all study groups (p=0.0002). We found no correlation between maternal leptin and cord leptin concentrations amongst the entire study population or according to racial grouping. There was a statistically significant (p=0.014) positive correlation between maternal weight and infant birth weight in white participants, but not in black participants. In contrast to other published data, in white infants there was no relationship between birth weight and cord blood leptin levels. However in black infants, there was a strong correlation between birth weight and cord blood leptin levels (p=0.0026).

Conclusions:

Maternal serum leptin levels strongly correlate with maternal weight in both racial groups. Leptin levels correlated positively with black infant birth weight but not with white infant birth weight. This differs from previously reported data and may be due to smaller sample size in the white infant subgroup. There do not appear to be racial differences in the concentrations of leptin in maternal serum or umbilical venous cord blood.

631

Does Maternal Milk Leptin Influence Fetal Serum Leptin Concentrations in the First 21 Days of Neonatal Life in the Rat? Elena Zambrano,[1] Claudia J Bautista,[1] Fernando Larrea,[1] Peter W Nathanielsz.[*2] *[1]Dept. Reproductive Biology, Instituto Nacional de Ciencias Medicas y Nutricion SZ, Mexico City, Mexico; [2]Center for Pregnancy and Newborn Research, The Univ. Texas Health Science Center at San Antonio, Dept. Ob/Gyn, San Antonio, TX, USA.*

OBJECTIVE. Associations have been published between offspring weight, offspring growth, and milk leptin concentration. In altricial species where an early postnatal leptin surge plays a critical role in maturation of brain appetitive centers brain, it is important to determine whether leptin responsible for this key maturational function is of fetal or maternal origin. We asked the question whether there is any correlation between rat serum and milk leptin concentrations on days 7, 14, and 21 of neonatal life in the rat. We also looked at effects of different nutritional restriction regimens. **METHODS.** Pregnant rats were assigned to control (C) (20% casein; CC) or a restricted (R) (10% casein; RR) isocaloric diet in pregnancy (P) (first letter) and lactation (L) (second letter). A third group received C and R in P and L, (CR). A fourth group received R in P and C in L (RC). At birth litters were adjusted to 12 pups. At 7.00 AM on PND 7, 14 and 21, pups were removed from their mother for 4 h after which mothers received 0.8 U oxytocin (ip); milk was expressed 15 min later. Pup serum was obtained at PND 7, 14 and 21. Milk leptin and pup serum leptin were measured by RIA. Data mean ± SEM; analysis by Pearson correlation. **RESULTS.** Milk leptin was not correlated with pup serum leptin on any individual group at day 7, 14 and 21 (p>0.05; Fig 1) or when all the groups and days were pooled (p=0.7). For both groups restricted during pregnancy (RR and RC) the highest pup leptin was at day 21, suggesting a delayed peak in leptin in pups undernourished prenatally. **CONCLUSION.** There is not correlation between milk leptin and pup serum leptin in neonatal rats. However, the normal post-natal rise in leptin was delayed in animals undernourished pre-natally. It is these groups that show obesity if allowed to eat ad libitum postnatally which may be due to altered appetitive control.

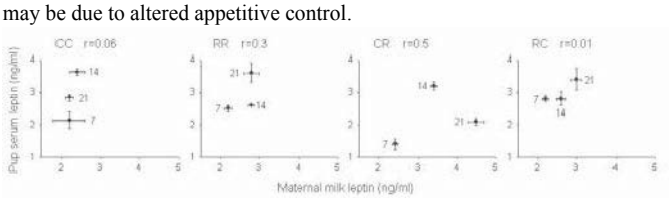


Fig. 1. Pup serum leptin as a function of maternal milk leptin on PND 7, 14 and 21 in the four groups exposed to different diets during P and L as described in the text. Mean ± SEM, n= 5 mothers or litters.

SATURDAY

632

Is There an Insensitive Early Post-Natal Period in Leptin Feedback on Appetitive Behavior in the Rat? Elena Zambrano,[1] Claudia J Bautista,[1] Lourdes Boeck,[1] Peter W Nathanielsz.*[2] [1]*Dept. Reproductive Biology, Instituto Nacional Ciencias Medicas Nutricion SZ, DF, Mexico;* [2]*Center for Pregnancy and Newborn Research, The Univ. Texas Health Science Center at San Antonio, Dept. Ob/Gyn, San Antonio, TX, USA.*

OBJECTIVE. Several studies show a surge of leptin **(L)** around post natal days (PND) 10 to 15 in rats. This surge is correlated with maturation of central nervous mechanisms that regulate appetite in later life. No studies have been conducted on the relationship of serum **L** and appetitive behavior in the neonatal period in the rat. **METHODS.** Pregnant rats were assigned to control (C) (20% casein; CC) or a restricted (R) (10% casein; RR) isocaloric diet in pregnancy (P) and lactation (L). Other groups received C and R in P and L, respectively (CR) or R in P and C in L (RC). At birth litters were adjusted to 12 pups. At 7.00 AM on PND 7, 14 and 21, pups were removed from mothers for 4h during which pups did not feed. Pups were weighed when returned to their mothers and again 1h later. At 2 PM pup serum was obtained for **L** measurement by RIA. Mean ± SEM; analysis by ANOVA and Pearson correlation. **RESULTS.** There was no correlation ($p>0.05$) between pup serum **L** and body weight gain after 1h of feeding (milk intake) on any individual day or when all PND were pooled (Table 1). Indeed the tendency of all the correlations was for milk intake to increase in the presence of increased pup serum **L**. Although food intake in RR was lowest on PND 21, RC was not different from CC. **CONCLUSION.** There is not correlation between serum **L** and appetitive behavior in the neonatal period in the rat.

	Group	7 d	14 d	21 d
Milk Intake (g/h)				
	CC	0.05 ± 0.02^{a}	0.27 ± 0.03^{a}	0.66 ± 0.09^{a}
	RR	0.13 ± 0.04^{ab}	0.15 ± 0.02^{b}	0.25 ± 0.05^{b}
	CR	0.08 ± 0.03^{a}	0.11 ± 0.02^{b}	0.43 ± 0.06^{ab}
	RC	0.20 ± 0.04^{b}	0.51 ± 0.04^{c}	0.64 ± 0.08^{a}
Serum Leptin (ng/ml)				
	CC	2.1 ± 0.3^{ab}	3.6 ± 0.2^{a}	2.8 ± 0.2^{ac}
	RR	2.5 ± 0.1^{a}	2.6 ± 0.1^{b}	3.6 ± 0.3^{b}
	CR	1.4 ± 0.2^{b}	3.2 ± 0.1^{ac}	2.1 ± 0.1^{a}
	RC	2.8 ± 0.1^{a}	2.8 ± 0.2^{bc}	3.4 ± 0.3^{bc}

Table 1. Milk intake (weight gain after 1h of feeding) and pup serum L on PND 7, 14 and 21 of progeny exposed to different diets during pregnancy and lactation as described in the text. Mean ± SEM, n = 5 litters. Data not sharing a letter are statistically different at the same age, $p<0.05$.

633

Alteration of Cord Lipids in Offspring of Mothers with Type I Diabetes. Scott M Nelson,[1] Dilys F Freeman,[1] Naveed Sattar,[2] Robert Lindsay.[2] (SPON: Jane E Norman). [1]*Reproductive & Maternal Medicine, University of Glasgow, Glasgow, United Kingdom;* [2]*BHF GCRC, University of Glasgow, Glasgow, United Kingdom.*

Objective: Maternal diabetes during pregnancy is associated with characteristic overgrowth of the fetus and increased risk of obesity and type 1 and 2 diabetes in the offspring. While increased maternal glucose appears central to increases in birth weight in type 1, type 2 and gestational diabetes, there has been interest in the potential role of over supply of lipids to the fetus as an additional factor in promoting fetal adiposity. We hypothesised that if upregulation of placental lipid transfer were key in promoting growth *in-utero* in offspring of type 1 diabetes mothers (OT1DM), fetal cord lipids would be higher and correlate with birth weight and fetal adiposity.

Methods: We have assessed cholesterol, high density lipoprotein-cholesterol (HDL-C), triglycerides (TG), very low density lipoprotein-cholesterol (VLDL-C), low density lipoprotein-cholesterol (LDL-C) and total non-esterified fatty acids (NEFA) in cord blood in controls and OT1DM. We have exmined the relationship of cord lipids to birth weight and measures of fetal adiposity and more broadly to maternal glycaemia (assessed by HbA1c) and to hormones in cord blood related to fetal carbohydrate metabolism, lipid metabolism and growth (insulin, insulin propeptides, leptin, adiponectin and insulin like growth factor-1).

Results: In a total of 140 OT1DM and 48 control offspring, maternal diabetes was associated with *lower* concentrations of HDL-C (OT1DM 0.62 ± 0.25 mmol/l; control 0.69 ± 0.18 mmol/l; P=0.05) and NEFA (OT1DM 0.18 [0.13 – 0.24] mmol/l; control 0.24 [0.18 – 0.33] mmol/l; P<0.0001). Cord lipids were unrelated to birthweight in OT1DM. Unexpectedly, IGF-1 was a strong predictor of HDL-C in controls (P=0.002) and OT1DM (p<0.001) and a negative predictor of TG in controls (P<0.001) and OT1DM (P=0.004). HDL-C was also independently determined by leptin in OT1DM (P<0.001), with TG determined by leptin (P=0.024) and insulin (P=0.003) in addition to IGF-1 in OT1DM.

Conclusion: Cord lipids, particularly NEFAs, are reduced in OT1DM, however, HDL-C, the principal fetal cholesterol does not correlate with fetal insulin or birthweight in OT1DM, but shows an unexpectedly strong, and novel association with IGF-1.

634

Visfatin Polymorphisms and Gestational Diabetes. Janet M Burlingame, Johann Urschitz, Kenneth Ward.* *Pacific Research Center for Early Human Development, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI, USA.*

Objective: There are ethnic differences in the incidence and morbidities of type 2 diabetes (T2DM) and gestational diabetes (GDM). Visfatin or Pre-B-cell colony enhancing factor 1 (PBEF1) is a cytokine that has been implicated in T2DM and GDM. Polymorphisms in the visfatin gene have also been associated with T2DM but have not been investigated in GDM. Visfatin is a particularly intriguing candidate gene because it is insulin-mimetic in visceral adipose tissue. Furthermore it is produced in placental membranes and is found in higher concentrations during pregnancy. We performed a candidate gene study to look for associations between visfatin single nucleotide polymorphisms (SNPs) and GDM in two populations.

Methods: Blood was collected from Caucasian and Asian women who met Coustan-Carpenter criteria for GDM and from ethnically matched non diabetic controls. DNA was extracted and real time PCR (*TaqMan Genotyping Assays, Applied Biosystems)* was used to screen for three visfatin non-coding SNPs, rs711438, rs6947766 and rs10447822. Genotype and allele frequencies were tested using chi-square contingency tests.

Results: Genotype and allele frequencies for the rs711438 SNP are outlined in the table below. Genotype frequencies were in Hardy-Weinberg equilibrium. The other two SNPs (rs6947766 and rs10447822) did not show any association with GDM. In the Caucasian population only, rs711438 genotype frequencies were significantly different in the GDM group (p=0.022). The GDM group showed an excess of the homozygous A genotype (p=0.006). The relative risk for GDM with the AA genotype was 1.9 (1.2-3.1, 95% CI).

Conclusion: In this first study to look at visfatin genetic polymorphisms as risk factors for GDM, we have demonstrated an allele that is significantly associated with GDM in a Caucasian population. The lack of association in the Asian population may reflect either their different mutational history of differences in the role of visceral adiposity between the groups. Future research will aim to find the biological basis for this genetic association and to find other DNA markers which might replace the current, inefficient glucola screens and allow more targeted interventions.

rs711438 Genotype and Allele Frequencies

	GG Genotype	GA Genotype	AA Genotype	A allele
Caucasian GDM (n=55)	11%	33%	56%	77%*
- Controls (n=191)	15%	49%	36%	60%
Asian GDM (n=17)	0%	18%	82%	91%
- Controls (n=51)	2%	12%	86%	92%

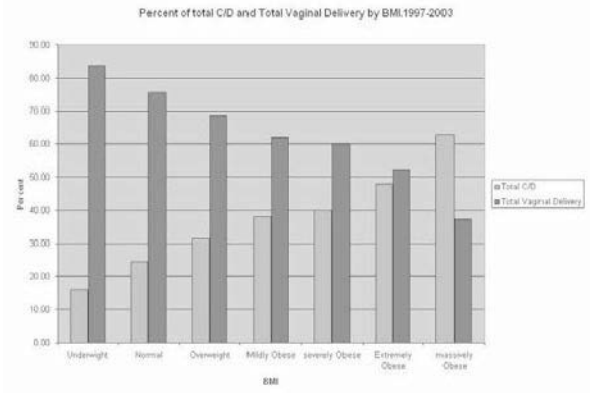
* p=0.002 with 95% CI

635

Does BMI Affect the Mode of Delivery in Diabetic Patients? Ana Hernandez, Daniel Barraez, Garry Finkle, Camille Kanaan, Jean-Claude Veille.* *Obstetrics Gynecology, Albany Medical Center, Albany, NY, USA.*

Introduction: Diabetics have an increase rate of complications during pregnancy. Diabetics also have larger babies and more operative deliveries leading to poorer obstetrical outcome. **Objective:** To determine if Body Mass Index [BMI] in Diabetics is contributor to the mode of delivery. **Material-Methods**: Data were tabulated from the Regional Perinatal Center (RPC) of the North Eastern region of New York State [NENY]. Data were available on 121,023 deliveries in hospitals in 20 counties from 1997-2005. **4,989** patients with either pre-gestational or gestational diabetes with no other co-morbidity and who delivered at term were identified. Patients were categorized into 6 groups based on BMI: Group1 = Underweight (BMI < 18.5 kg/m^2) [n= 105]; Group 2: Normal (BMI=18.5-24.9 kg/m^2)[n=1,476]; Group 3: Overweight (25-29.9kg/m^2)[n=1,269]; Group 4: Mildly Obese (30-34.9kg/m^2)[n = 963]; Group 5: Severely Obese (35-39.9 kg/m^2) [n = 639]; Group 6: Extremely Obese(40-44.9kg/m^2)[n = 345] and Group 7: Massively Obese (>45kg/m^2)[n=191].

Results: The overall rate for Cesarean Delivery for the groups were as follow: Group 1 =16.19%, 2 = 24.39%; 3 = 31.28%; 4= 38.01%; 5=40.06%; 6= 47.77%; 7= 62.78%. The graph below illustrate a linear relationship between BMI and incidence of C/D in this population independently of other co-morbidities [Fig. 1]. This increase was due to an increase in birth weights [BW] among the groups ($p < 0.0001$)[Fig. 2].**Conclusions:** 1) Obesity and Diabetes increase the risks for C/D by 5.2 [OR 3.8-7.1, $p < 0.0001$]; 2) This increase is secondary to the presence of larger fetuses in diabetic patients; 3) Diabetes increases the risks for C/D independently of BMI by 1.5 [OR 1.2-1.7; $p < 0.0001$].



636

TCF7L2 Polymorphism Is Associated with Gestational Diabetes. Johann Urschitz, Janet M Burlingame, Kenneth Ward.* *Pacific Research Center for Early Human Development, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI, USA.*

Objective: Recently, several studies have associated the transcription factor 7-like 2 gene (TCF7L2) with increased risk of type 2 diabetes (T2DM) and its progression primarily in Caucasian populations. TCF7L2 is expressed in pancreatic beta-cells and in many other human cell types and is thought to be involved in glucose homeostasis. As risk factors are similar between T2DM and gestational diabetes (GDM) we sought to investigate a possible association of TCF7L2 (rs12255372) with GDM.

Study Design: Blood was collected from women who met Coustan-Carpenter criteria for GDM and ethnically matched non diabetic controls. DNA was extracted and a candidate gene association study was performed using real time PCR technology (TaqMan, Applied Biosystems) to screen maternal DNA. Comparisons were made for the rs12255372 single nucleotide polymorphism (SNP) located in intron 3 of the TCF7L2 gene.

Results: Chi square contingency tests were used to analyze genotype and allele frequencies in controls and GDM affected pregnancies. The findings for the rs12255372 SNP are outlined in the table below.

Genotype counts showed an excess of homozygotes for the TT allele ($p=0.007$). TT individuals have an increased risk of GDM with a relative risk of 2.6 and confidence limits of 1.3-5.3

Conclusion: This is the first study to show that a common polymorphism in the TCF7L2 gene associated with type 2 diabetes is also associated with GDM in Caucasian women. Future research will aim to find the biological basis for this genetic association and to find other DNA markers. These findings may someday allow for a replacement of the current, inefficient glucola screens and more targeted interventions.

Incidence of rs12255372

	TT Genotype	TC Genotype	CC Genotype	C Allele Frequency
Caucasian GDM	12	16	37	70%
Caucasian Control	14	79	109	74%

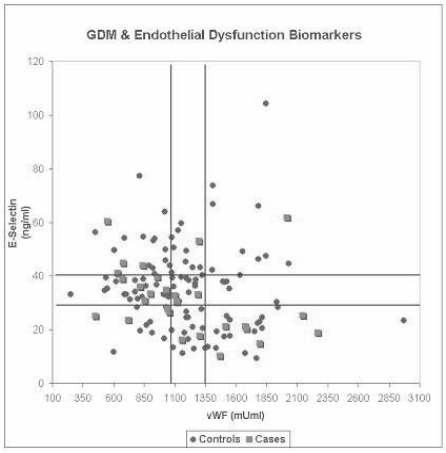
637

E-Selectin and Von Willebrand Factor, Potential Biomarkers for Gestational Diabetes Mellitus. Jose A Rauh-Hain,[2] Hector Tamez,[2] Ravi I Thadhani,[2] Jeffrey Ecker.*[1] *[1]Division of Maternal-Fetal Medicine, Massachusetts General Hospital, Boston, MA, USA; [2]Clinical Research in Nephrology, Massachusetts General Hospital, Boston, MA, USA.*

Background: Gestational Diabetes Mellitus (GDM) is one of the most common medical complications of pregnancy. In the U.S. approximately 150,000 to 200,000 women are diagnosed annually with GDM. Elevated plasma biomarkers of endothelial dysfunction are independent predictors of type 2 diabetes, and they may also predict GDM.

Objective: To determine whether biomarkers reflecting endothelial dysfunction (E-Selectin, Von Willebrand Factor [vWF]) predict the development of GDM.

Methods: We performed a nested case–control study within the Massachusetts General Hospital Obstetrical Maternal Study (MOMS). A total of 28 cases of GDM and 133 controls were consecutively chosen. Blood samples were obtained between 15-18 weeks of gestation. GDM was diagnosed by a 100-g oral glucose tolerance using standard criteria. E-Selectin was measured using commercially available ELISA, and vWF antigen was determined on a STA Clot analyzer. Students t test and Wilcoxon rank sum tests were used as appropriate. We also divided the distributions of vWF and E-Selectin into tertiles, according to the distribution in controls,



and used regression models to estimate the risk of GDM.

Results: There was no overall difference in E-Selectin and vWF mean levels at 15-18 weeks between women who subsequently developed GDM and those that did not: E-Selectin, 30.8 +/- 11.94 vs. 35.2 +/- 16.009 ng/ml ($p=.08$), and vWF 1202 +/- 480 vs. 1209 +/- 414 mUml ($p=0.47$), respectively. The adjusted odds ratio for development of GDM in the top tertile versus the bottom two tertiles were 1.08 for Von Willebrand Factor (95% CI, 1.0-1.1) and 0.92 for E-Selectin (95% CI, 0.79-1.42).

Conclusion: Plasma concentrations of E-Selectin and Von Willebrand Factor are not increased between 15-18 weeks of gestation in women who later developed GDM.

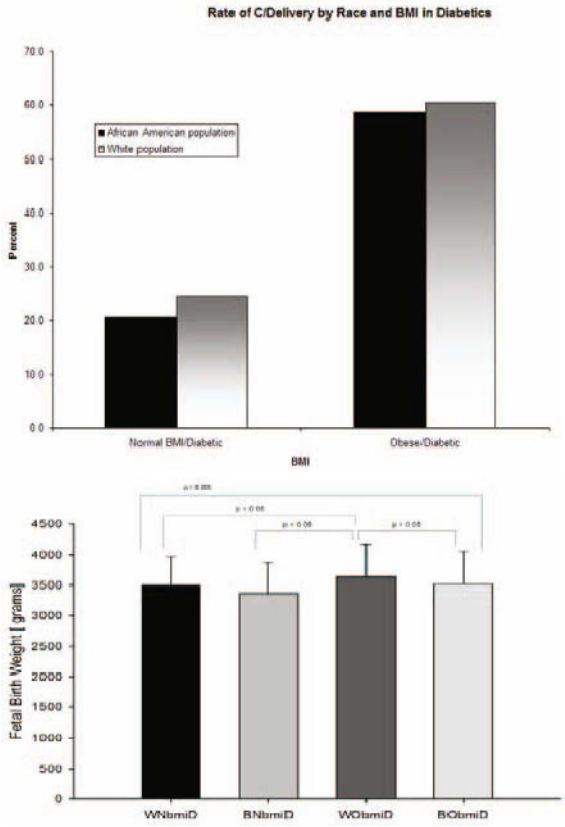
638

Does Race and BMI Affect the Incidence of C/D in Diabetics Patients? Rashida Randeree, Daniel Barraez, Audoen Maddox, Camille Kanaan, Jean-Claude Veille.* *Ob Gyn, Albany Medical Center, Albany, NY, USA.*

Introduction: Body Mass Index [BMI] and Diabetes influence the mode of delivery. We investigated the effect of BMI, race and the presence of Diabetes significantly affect the mode of delivery. **Objective:** To determine if Obese Diabetic Whites [W] had a different rate of Cesarian Deilvery [C/D] than Obese Diabetic African American [B] when classified by BMI. **Material-Methods**: Data were tabulated from the Regional Perinatal Center (RPC) of the North Eastern region of New York State [NENY]. Data were available on 121,023 deliveries in hospitals in 20 counties from 1997-2005. A total of **4,641** diabetic patients had both pre-pregnant BMI and race tabulated. These diabetic patients were categorized into a race category [White vs Black] and divided into 2 groups based on BMI: Group **1** Normal BMI/Diabetics [N] (BMI=18.5-24.9 kg/m^2)[W=1251; B=63]; **2**: Obese/Diabetes [O](BMI > 25 kg/m^2) [W= 3028; B =206]. **Results:** The overall rate for C/D was: Group [1]: W=24.3%; B=20.6%; [2]: W= 60.6%; B= 58.5%; The graph below illustrate a linear relationship between BMI and incidence of C/D between W and B [Fig. 1]. Obese diabetic W or B had a greater rates of C/D than non-obese W

SATURDAY

or B diabetes. This increase in C/D was due to an increase in the birth weights in the W population (p <0.05)[Fig. 2], however fetal weight cannot explain this diference in C/D in the B population as we found no difference in birth weights between B diabetics [N] vs [O].**Conclusions:** 1) Among diabetics patients, obesity was a significant contributor to operative delivery in both W and B. 2) Diabetic patients who had a normal BMI had no significant increase in C/D regardless of race.3) This increase in C/D could be explained by a greater birth weight only in W patients. The reason(s) for this difference needs to be further explored.



639

Impact of Maternal Obesity on Growth and Pancreatic Function in the Fetal Sheep. Stephen P Ford,[*1,2] Myrna M Miller,[1,2] Bret W Hess,[1,2] Gary E Moss,[1,2] Peter W Nathanielsz.[*1,3] *[1]Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA; [2]Department of Animal Science, University of Wyoming, Laramie, WY, USA; [3]Center for Pregnancy and Newborn Research, Department of Obstetrics & Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: Maternal obesity correlates with increased offspring weight and a potential to change body composition, and developing tissue and organ function. **Hypothesis:** Maternal obesity causes fetal macrosomia, with asymmetric increases in fetal tissue growth and results in changes in fetal pancreatic development and function. **Methods:** Ewes were assigned to a control (C, 100% of NRC recommendations, n=5) or obesogenic (OB, 150% of NRC, n=6) diet from 60 days before to 75 days after conception when animals were euthanized. Paraffin-embedded fetal pancreatic tissue sections were incubated with guinea pig anti-porcine insulin or mouse anti-glucagon antibodies at 4° C overnight, then with fluorescent labeled 2° antibodies Rhodamine labeled goat anti-guinea pig or AlexaFluor 488 labeled goat anti-mouse for 60 min at 22° C. Insulin and glucagon positive cell number was determined/unit area of pancreatic tissue. **Results:** OB ewes increased (P<0.05) their body weight by ~50%, while C ewes increased their body weight only ~7% from diet initiation until necropsy. Fetuses from OB ewes were heavier than those from C ewes (374 ± 10 vs. 268 ± 12 g, P<0.05). Although all organs were heavier (P<0.05) in fetuses from OB vs. C ewes, only pancreatic weight was increased relative to fetal body weight (0.05 ± 0.01 vs. 0.12 ± 0.01 %, P<0.05). Numbers of insulin positive cells/unit pancreatic area were 50% greater (P<0.06) in fetuses from OB vs. C ewes, while numbers of glucagon positive cells were similar. Further, both the concentrations of glucose and insulin were elevated (P<0.05) in the blood of OB vs. C ewes (65.5 ± 6.6 vs. 52.1 ± 3.5 mg/dL and 25.0 ± 9.0 vs. 4.8 ± 1.6 uIU/ml, respectively) and fetuses (40.7 ± 4.2 vs. 26.2 ± 2.3 mg/dL and 5.8 ± 0.8 vs. 1.6 ± 0.1 uIU/ml, respectively) on day 75. **Conclusions:** The increase in blood insulin concentrations of fetuses gestated by OB vs. C ewes may result from increased maternal glucose delivery into the fetal compartment, or may reflect developing insulin sensitivity. The observed acceleration of pancreatic development in OB vs. C fetuses may alter pancreatic function in later life. NIH INBRE 1P20RR16474.

640

Early Pregnancy Metabolic Environment in Obese Women Predicts Birth Weight. Nina Jansson,[1] Anna Nilsfelt,[1] Lena Rossander-Hulthen,[2] Theresa Powell,[1] Thomas Jansson.[1] (SPON: Leslie Myatt). *[1]Perinatal Center, Department of Physiology, Institute of Neuroscience and Physiology, Göteborg University, Göteborg, Sweden; [2]Department of Metabolism and Cardiovascular Research, Institute of Medicine, Göteborg, Sweden.*

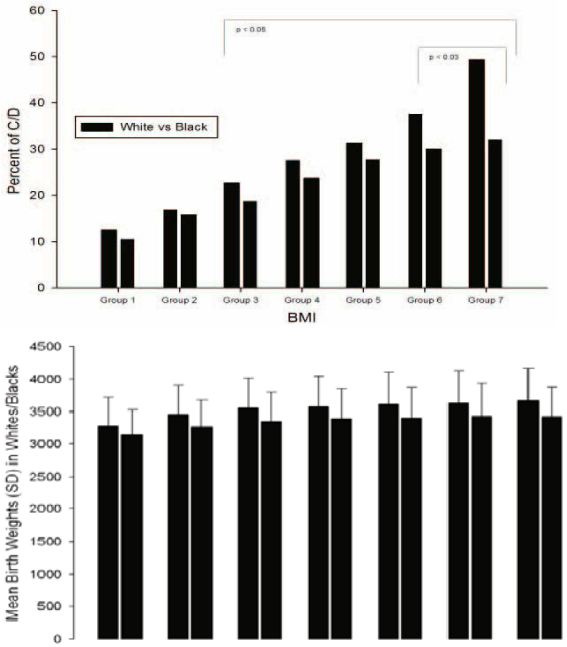
The prevalence of overweight and obesity in pregnancy is increasing rapidly. Fetal overgrowth, a common outcome in pregnancies of women with increased BMI, is associated with traumatic birth injuries and an increased risk to develop obesity, diabetes and hypertension in childhood and later in life. The underlying mechanisms are currently unknown but fetal overgrowth is likely to be related to the altered maternal metabolism of the obese woman and increased nutrient delivery to the fetus. We tested the hypothesis that maternal metabolic biomarkers and diet in overweight and obese women are distinctly different in early pregnancy and that these alterations are related to birth weight. Method: Forty-seven women with BMI between 17 and 44 were recruited in weeks 8-12 of gestation, detailed dietary analysis was carried out and blood samples were obtained. Birth size and outcomes were recorded at delivery. Results: As expected, early pregnancy BMI was significantly correlated with birth weight (p<0.01). Maternal BMI was positively correlated with serum insulin (p<0.001), leptin (p<0.001), LDL (p<0.002), and negatively correlated with serum HDL (p<0.05) and adiponectin (p<0.05). Of the serum markers measured, leptin (p<0.001) and insulin (p<0.001) levels in early pregnancy were positively correlated with birth weight. Of the dietary variables measured, high caloric intake (p<0.02) in early pregnancy was associated with an increased size at birth. Further examination of the dietary components revealed that first trimester intake of protein (p<0.05), monounsaturated fatty acids (p<0.04) and polyunsaturated fatty acids (p<0.02) were positively correlated with birth weight. Conclusions: This is the first study to report metabolic biomarkers and dietary intake in early pregnancy of women with differing BMI and how they correlate to birth weight. Overweight and obese women had markedly altered serum levels of hormones and nutrients and their diet was characterized by a high caloric intake. High nutrient availability in first trimester may set the fetal growth trajectory for rapid growth in late pregnancy. The hormones leptin and insulin, which were elevated in the obese women, are known to stimulate placental nutrient transporters and may contribute to fetal overgrowth.

641

Does Race Affect the Mode of Delivery in Obese Mothers? Daniel Barraez, Audoen Maddock, Garry Finkell, Camille Kanaan, Jean-Claude Veille.[*] *Ob Gyn, Albany Medical Center, Albany, NY, USA.*

Introduction: Body Mass Index [BMI] influences the mode of delivery. We investigated whether race affected the mode of delivery in patients with different BMI. **Objective:** To determine if Whites [W] had a different rate of operative delivery than African American [B] when classified by BMI. **Material-Methods**: Data were tabulated from the Regional Perinatal Center (RPC) of the North Eastern region of New York State [NENY]. Data were available on 121,023 deliveries in hospitals in 20 counties from 1997-2005. A total of **94,636** patients had both pre-pregnant BMI and race tabulated. None had other co-morbidity. Patients were categorized into a race category [White vs Black] and divided into 7 groups based on BMI: Group**1** = Underweight (BMI < 18.5 kg/m^2) [W= 3749; B= 281]; **2:** Normal (BMI=18.5-24.9 kg/m^2)[W=46,970; B= 2730]; **3**: Overweight (25-29.9kg/m^2)[W=20,718; B= 1687]; **4**: Mildly Obese (30-34.9kg/m^2)[W=9794; B= 920]; **5**: Severely Obese (35-39.9 kg/m^2) [W= 4410; B=431]; **6**: Extremely Obese(40-44.9kg/m^2)[W=1815; B= 180] and **7**: Massively Obese (>45kg/m^2)[W=833; B= 118]. **Results:** The overall rate for Cesarean Delivery was: Group [1] : W = 12.5 %; B=10.7%; [2]: W= 17%; B= 15.8%;[3]:W=22.7%; B= 18.9%; [4] W= 27.7%; B= 23.7%; [5]: W= 31.3%; B =27.8%; [6]: W= 37.7%; B= 30%; [7]: W= 49.3%; B= 32.2%. The graph below illustrate a linear relationship between BMI and incidence of C/D between W and B [Fig. 1]. Obese W had a greater rates of C/D than obese B. This increase was due to an increase in the birth weights in the W population (p <0.01)[Fig.

2].**Conclusions:** 1) Among obese patient, B had less incidence of operative deliveries than W; 2) This was due to a significant larger BW of babies in W; 3) Being W and massively obese increases the chances of operative delivery by 2 fold [OR= 2.05; 95% CI 1.34-3.17; p < 0.0003].



642

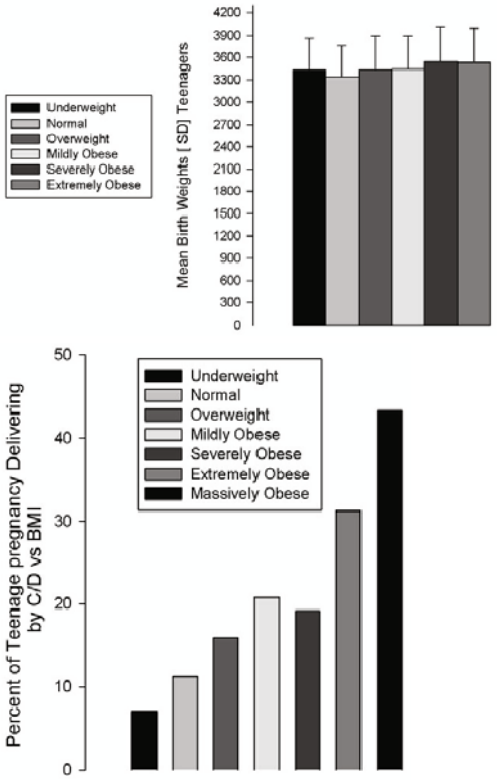
Fetal Steroid Changes Associated with Maternal Obesity in the Sheep. Stephen P Ford,[*1,2] Myrna M Miller,[1,2] Bret W Hess,[1,2] Gary E Moss,[1,2] Peter W Nathanielsz.[*1,3] *[1]Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA; [2]Department of Animal Science, University of Wyoming, Laramie, WY, USA; [3]Center for Pregnancy and Newborn Research, Department of Obstetrics & Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: Steroid hormones are associated with long-term organizational effects during development. Prenatal glucocorticoid overexposure appears to program adverse effects on blood pressure, metabolism and the HPA axis, while elevated prenatal testosterone exposure is linked to reproductive dysfunction. **Objective:** Utilizing our newly developed sheep model of maternal obesity, we evaluated its impact on fetal cortisol and testosterone concentrations at midgestation. **Methods:** Ewes were assigned to a control (C, 100% of NRC recommendations, n=5) or obesogenic (OB, 150% of NRC, n=6) diet from 60 days before to 75 days after conception when ewes were anesthetized. Following collection of maternal jugular and umbilical venous blood, fetuses were euthanized by exsanguination, and ewes were euthanized by an overdose of sodium pentobarbital. Serum was stored at -80ºC until assayed for cortisol and testosterone by RIA (Diagnostic Products, Los Angeles, CA). **Results:** OB and C ewes increased their body weights by ~50% and ~7%, respectively, from diet initiation until necropsy. Fetuses from OB ewes were heavier than those from C ewes (374 ± 10 vs. 268 ± 12 g, P<0.05). Maternal blood concentration of cortisol were greater for OB vs. C ewes (31.8± 5.9 vs. 3.6 ± 0.8 ng/ml; P<0.05), and was greater in the blood of their fetuses (7.7 ± 2.1 vs. 3.8 ± 0.8 ng/ml; P<0.05), regardless of fetal sex. Male fetuses exhibited greater blood concentration of testosterone than female fetuses regardless of dietary treatment (0.47 ± 0.11 vs. 0.04 ± 0.01 ng/ml, P<0.05). While blood concentrations of testosterone were similar for female fetuses from OB and C ewes (0.05 ± 0.01 and 0.04 ± 0.02 ng/ml), testosterone concentrations of male fetuses from OB ewes were greater than those from C ewes (0.83 ± 0.17 vs. 0.26 ± 0.13 ng/ml, P<0.05). **Conclusions:** These data demonstrate that maternal obesity elevates fetal blood cortisol, which may result from increased uptake of elevated maternal cortisol or de novo synthesis. Further, testosterone concentrations were elevated in the blood of male fetuses from OB vs. C ewes and may impact their reproductive function in later life. NIH INBRE 1P20RR16474.

643

Are Obese Teenagers at Greater Risk for Operative Deliveries? Daniel Barraez, Ana Hernandez, Garry Finkle, Camille Kanaan, Jean-Claude Veille.[*] *Ob Gyn, Albany Medical Center, Albany, NY, USA.*

Introduction: Teenagers have an increase rate of complications during pregnancy. Pregnant obese teenagers may have large babies and more operative deliveries. as is associated with poor obstetrical outcome. **Objective:** To determine if Body Mass Index [BMI] alone in "healthy" teenagers is a significant contributor to the mode of delivery. **Material-Methods**: Data were tabulated from the Regional Perinatal Center (RPC) of the North Eastern region of New York State [NENY]. Data were available on 121,023 deliveries in hospitals in 20 counties from 1997-2005. 9,252 teenagers with NO significant co-morbidity, who delivered at term and who had no other co-morbidity such as diabetes and/or hypertensive disorders. Patients were categorized into 6 groups based on BMI: Group1 = Underweight (BMI < 18.5 kg/m^2) [n= 860]; Group 2: Normal (BMI=18.5-24.9 kg/m^2)[n=5,418]; Group 3: Overweight (25-29.9kg/m^2)[n=1,890]; Group 4: Mildly Obese (30-34.9kg/m^2)[n = 703]; Group 5: Severely Obese (35-39.9 kg/m^2) [n = 255]; Group 6: Extremely Obese(40-44.9kg/m^2)[n = 96] and Group 7: Massively Obese (>45kg/m^2)[n=30]. **Results:** The overall rate for Vaginal Delivery for the groups were as follow: Group 1 =92.9%, 2 = 88.7%; 3 = 84.1%; 4= 79.2%; 5=80.8%; 6= 68.75%; 7= 56.7%. The graph below illustrate a linear relationship between BMI and incidence of C/D in this population independently of other co-morbidities [Fig. 1]. This increase was not due to an increase in birth weights [BW] among the groups [Fig. 2].**Conclusions:** 1) Obese Teenagers are also at a significantly greater risk of being delivered by C/D if their BMI is high; 2) This is independent of diabetes or hypertensive disorders; 3) Fetal weight does not seem to be significantly related to maternal pre-pregnant weight even if the mother was underweight at the onset of the pregnancy.



644

Obesity Increases Incision to Delivery Time for Patients Undergoing Repeat Cesarean Section. Courtney J Goodwin,[1] Nancy A Hueppchen,[1] Frank Witter,[1] Barbara Stoelinga,[2] Jessica L Bienstock.[1] (SPON: Ernest Graham). *[1]GYN/OB, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]School of Medicine, Erasmus University, Rotterdam, Netherlands.*

Objective: It is well known that previous c-section increases both the operative time and the complexity of the surgery for patients undergoing repeat c-sections. It is also known that surgical procedures on obese patients are often more technically challenging. We sought to explore the relationship between maternal obesity and its affects on patients undergoing repeat c-section when compared to normal weight controls and to obese patients who were undergoing primary c-sections.

SATURDAY

Study design: We performed a retrospective cohort study of all term patients meeting BMI criteria who were delivered by c-section utilizing regional anesthesia between December 2004 and June 2006. Normal weight patients were defined as having a BMI between 18.5 and 24.9. Obese patients were defined as having a BMI > 30. Maternal records from a single institution were reviewed. The student's T test was used for statistical analysis.
Results: There were 112 obese (54 primary and 58 repeat c-section) and 142 normal weight (85 primary and 57 repeat c-section) patients identified. Patients undergoing repeat c-section had significantly longer incision to delivery times compared to those undergoing primary c-section regardless of BMI. BMI did not contribute to incision to delivery time for patients undergoing primary c-section. However, obese patients undergoing repeat c-section saw a 35% increase in incision to delivery time when compared to normal weight controls (Table). Obese patients undergoing repeat c-section had an 82% increase in incision to delivery time when compared to obese patients undergoing primary c-section (10.4 vs. 18.9 min). In contrast, normal weight patients saw only a 42% increase in operative time (9.85 vs. 14.0 min).
Conclusion: Obesity is associated with a significantly longer incision to delivery time for patients undergoing repeat c-section. This longer incision to delivery interval should be considered when a clinical situation dictates that an obese patient may need to undergo a repeat c-section.

Incision to Delivery Time

	Normal Weight	Obese	p-value
Primary C/S	9.85 min	10.4 min	0.4
Repeat C/S	14.0 min	18.9 min	0.002
p-value	<0.001	<0.001	

645

Demographic and Biological Predictors of Gestational Weight Gain. Alison M Stuebe,[1] Thomas F McElrath,[1] Ravi Thadhani,[2] Jeffrey L Ecker.*[2]
[1]*Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA;* [2]*Massachusetts General Hospital, Boston, MA, USA.*
Background: Because prior work suggests an association between high insulin concentrations in early pregnancy and excess gestational weight gain, we examined such associations in a well defined cohort.
Methods: We studied a prospective cohort of 457 women enrolled in the MGH Obstetrical Maternal Study who delivered after 37 weeks´ gestation. Fasting serum samples collected at 16-18 weeks gestation were analyzed to determine insulin levels and homeostasis model assessment (HOMA). We abstracted baseline (first trimester first visit) weights and weight gain from the electronic medical record and classified gain according to Institute of Medicine guidelines. We used simple and multivariable regression to examine associations among these variables.
Results: Contrary to others' earlier work, we found in univariate analyses that gestational weight gain was inversely associated with early pregnancy fasting insulin levels (4th quartile: -3.38 lbs, 95% CI -5.61 to -0.95). BMI confounded this association. In multivariable analysis, we found no association between insulin quartile (p .76) or HOMA quartile (p .63) and gestational weight gain, adjusting for BMI, parity, maternal age, white race, and smoking status. In logistic regression, weight gain exceeding IOM guidelines was independently associated with white race (OR 1.85, 1.10-3.12) and overweight (OR 3.57, 2.03-6.28) or obese BMI (OR 2.04, 1.13-3.70), adjusting for age, parity, smoking status and insulin quartile.
Conclusion: In our cohort, insulin homeostasis in early pregnancy was not independently related to gestational weight gain. White race and overweight or obese BMI may identify women at increased risk of excessive gain.

Gestational Weight Gain (lbs)

	Parameter Estimate (95% CI)	p (partial F test)
Fasting insulin 16-18 wks		0.76
Q1	0 (ref)	
Q2	-0.156 (-2.86 to 2.55)	
Q3	1.06 (-1.66 to 3.77)	
Q4	0.67 (-2.39 to 3.73)	
Age (yrs)	-0.43 (-0.62 to -0.23)	<0.0001
Parity		0.003
0	0 (ref)	
1	-2.31 (-4.45 to 0.17)	
2+	-5.00 (-7.97 to -2.02)	
BMI		<0.0001
< 19.8	-1.02 (-4.13 to 2.09)	
19.8 - 25.9	0 (ref)	
26.0 - 29.0	-2.41 (-5.11 to 0.28)	
> 29.0	-7.53 (-10.38 to -4.67)	
White race	3.2 (0.92 to 5.52)	0.007
Smoking	-2.40 (-7.80 to 3.00)	0.38

646

Is BMI "Alone" a Predictive of the Mode of Delivery? Daniel Barreaz, Anna Hernandez, Garry Finkle, Camille Kanaan, Jean-Claude Veille.* *Obstetrics and Gynecology, Albany Medical College, Albany, NY, USA.*
Introduction: Body Mass Index [BMI] is associated with poor obstetrical outcome. **Objective:** To determine if the the degree of obesity [BMI] alone is a significant contributor to the mode of delivery. **Material-Methods**: Since 1997, the Regional Perinatal Center (RPC) has tabulated and compiled perinatal outcome data and trends throughout the North Eastern region of New York State [NENY]. Data were available on 121,023 deliveries in hospitals in 20 counties from 1997-2005. From this large group we elected to study only patients who delivered at term and who had no other co-morbidity such as diabetes and/or hypertensive disorders. Patients were categorized into 6 groups based on BMI: Group1 = Normal (BMI=18.5-24.9 kg/m^2)[n=52,713]; Group2: Overweight (25-29.9kg/m^2)[n=23,503]; Group 3: Mildly Obese (30-34.9kg/m^2)[n = 11,163]; Group 4: Severely Obese (35-39.9 kg/m^2) [n = 5,031]; Group 5: Extremely Obese(40-44.9kg/m^2)[n = 2,065] and Group 6: Massively Obese (>45kg/m^2)[n=982]. **Results:** The overall rate for Ceasarean Delivery [C/D] for the entire population was 21.05%; primary C/D [PC/D]= 12.4%; Repeat C/D[RC/D] = 8.7%. The total C/D rate for subjects with a normal BMI was 17% vs 22.5% for subjects with a BMI > 30.The table below illustrate a linear relationship between BMI and incidence of C/D independently of other co-morbidities. This increase was not due to an increase in birth weights [BW] among the groups.**Conclusions:** 1) BMI is associated to a decrease in VB, increase in C/D, decrease in R C/D, and decrease in VBAC; 2) This occurs independently of maternal co-morbidity such as diabetes or hypertension; 3) Although BW were higher in the obese groups, this was not statistically significant; 4) The overall difference in BW was only 200gms between the Normal group and the Massively obese group; 5) BW does not seem to be influenced by maternal weight but may be related to other variable co-morbidity such as diabetes.

Method of Delivery by BMI

Groups	VD	Total C/D Rate	Primary C/D	Repeat C/D	VBAC
1[BMI 18.5-24.9]	79%	17.1%	10.9%	6.2%	3.9%
2 [BMI 25-29.2]	73.3%	22.4%	12.9%	9.5%	4.24%
3 [BMI 30-34.9]	68.2%	27.4%	14.9%	12.5%	4.5%
4[BMI 35-39.9]	65.0%	30.9%	16.2%	14.7%	4.03%
5 [BMI 40-44.9]	58.6%	37.2%	18.3%	18.9%	4.1%
6 [BMI > 45]	48.9%	47.3%	23.5%	23.8%	3.7%

Birth Weights in gms [X +/- SD]

Normal	3442.5	463
Overweight	3529.7	505
Mildly Obese	3555.0	525
Severely Obese	3588.8	493
Extremely Obese	3606	578
Massively Obese	3635.6	495

647

Pregnancy Outcome in Obese Primiparous Pregnant Women Recruited to the Vitamins in Pre-Eclampsia Trial. Lucilla Poston,* Paul T Seed, Daghni Rajasingham, Annette Briley, Andrew H Shennan. *Maternal & Fetal Research Unit, Division of Reproduction & Endocrinology, King's College London, United Kingdom.*
Objectives. We have investigated pregnancy outcome, and biochemical variables in pre-randomisation plasma samples (14-22/40) in 769 primiparous obese pregnant women in the Vitamins in Pre-eclampsia trial (UK) (mean BMI 36 Kg/m^2, range 30-65; mean age 30; range 17-44). None of the outcomes reported was influenced by antioxidant supplementation.
Methods. Associations with BMI and outcome variables were interrogated by regression analysis with robust standard errors. Plasma lipids, malondialdehyde, uric acid, vitamin C, α and γ tocopherol were determined by standard methods.
Results. Within this primiparous obese cohort, the incidence of pre-eclampsia was related to BMI. Preterm delivery (<37/40) was high (11.7% CI 9.5-14.2) as was the caesarean section rate (42.5% CI 39.0-46.1), which increased with BMI. Mean birthweight was within normal range (3.36kg, CI 3.31-3.41) and was not associated with BMI. 12.3% of babies were SGA (<5th centile) and 9.7% had macrosomia (>95th centile). BMI was related to plasma concentrations of uric acid (r=0.144, p<0.0005), γ tocopherol (r=0.143, p= 0.004) and negatively with HDL cholesterol (r= -0.098, p= 0.045).

Conclusions. Primiparous obese women were at risk of SGA delivery as well as pre-eclampsia, preterm birth, macrosomia and caesarean section. Parity should be considered in studies of obese women.

Association with BMI and outcome in primiparous women with BMI >30

Outcome	Effect associated with a 1kg/m² change in BMI		
	Odds Ratio	95%CI	p
Pre-eclampsia	1.08	1.04-1.12	<0.0005
SGA	0.99	0.94-1.05	0.785
Macrosomia	1.04	1.00- 1.09	0.048
Caesarean Section	1.05	1.02-1.08	0.002
Assisted delivery	0.97	0.93-1.01	0.127
SVD	0.97	0.94-1.00	0.041
	Mean difference		
Maternal Inpatient nights	0.19	0.10-0.28	<0.0005
Birthweight (g)	-0.50	-12.6 to 11.6	0.935
Birthweight centile	0.48	-0.17 to 0.98	0.058

648

Does One Hour Glucose Challenge Test Predict the Dynamics of Obstetric Outcome in Gestational Diabetes? Oormila Kovilam, Annette Bombrys, Rose Maxwell, Lori Packard, Sahay Rashmi. (SPON: Kenneth E Clark). *Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

One hour glucose challenge test (GCT) is an initial reflection of the metabolic alteration of gestational diabetes. This index value is not currently applied to predict maternal and fetal morbidity associated with gestational diabetes (GDM). Our objective was to test the hypothesis that obstetric morbidity is directly proportional to the screening one hour GCT value in patients with GDM.

Demographic, obstetric, and neonatal data from 224 consecutive gestational diabetics who were managed with tight glycemic control (<90 mg/dl fasting and <120 mg/dl 1 hour postprandial) and delivered at the University Hospital were collected. These cases were grouped according to their one hour GCT values. These groups were compared for body mass index (BMI), preterm delivery (PTD), cesarean delivery (CD), birth weight (BW), and neonative intesive care unit (NICU) stay. Data analysis were performed with student t-test and chi square.

Mean and percents for one hour GCT and adverse obstetric outcomes are presented in the table.

In well controlled gestational diabetics, the degree of one hour glucose values did not indicate adverse obstetric outcome. This demonstrates that strict glycemic control can optimize the outcomes irrespective of the initial GCT value.

1 Hour GCT and Obstetric Outcomes

Glucose Range (mg/dl)	BMI	PTD (%)	CD (%)*	BW (g)	NICU Days
130-150	34.8±7.8	27	44	3330.2±660.1	8.1±6.2
151-180	34.8±9.1	35	57	3132.5±923.4	8.4±12.3
181-200	35.6±7.6	29	19	3256.5±659.7	11.3±5.9
>201	36.1±10.3	36	39	2996.6±745.4	17.1±13.6

* p SIGNIFICANT FOR <0.05

649

Case-Control Evaluation of the Effectiveness of Betamethasone for Prevention of Neonatal Morbidity and Mortality in Preterm Twin and Singleton Gestations. Leah Battista,[1] Deborah A Wing,[*1] Kim C Walters,[1] Cristiane Hagemann,[1] Pamela J Rumney,[1] Wright Elysia.[2] *[1]Obstetrics and Gynecology, University of California, Irvine School of Medicine, Orange, CA, USA; [2]Pyschiatry, University of California, Irvine School of Medicine, Orange, CA, USA.*

Objective: To compare the effectiveness of antenatal betamethasone for the prevention of neonatal morbidity and mortality in preterm twin and singleton gestations.

Materials and Methods: Case-control study comparing the outcomes of twin and singleton neonates born to women who received betamethasone for risk of preterm delivery in a university-affiliated, community-based tertiary care center between 1997 and 2005. Cases were identified from computerized clinical care and pharmacy databases, and were matched to controls based on gestational age at delivery and neonatal gender. Comparisons were made between the first-born of the twins and singleton controls. Only those women delivering between 24 and 34 weeks' gestation were included. Sixty cases and 60 controls were identified.

Results: There were no differences in the twins compared to the singletons in maternal demographics (height, weight, BMI or ethnicity) or gestational age at delivery. There were no differences between the groups in birth weights (1434.5±466.5 vs. 1481.3±573.6g), head circumference at delivery (28.3±2.7 vs. 27.4±3.4 cm), Apgar scores at 1 and 5 minutes, need for mechanical ventilation (49.1% vs. 56.7%), number of days on ventilator (16.9±17.7 vs. 21.6±18.3), or total NICU days (46.8±31.6 vs. 42.0±32.8). Intraventricular hemorrhage (IVH) (13.8% vs. 21.0%, p<0.05), necrotizing enterocolitis (NEC) (12.1% vs. 16.7%, p<0.05), and neonatal deaths (1.7% vs. 6.7%, p<0.05) occurred more frequently in controls.

Conclusions: Differences in the neonatal outcomes of IVH, NEC and neonatal death occurred more frequently in singletons than in twins in this case-control investigation evaluating the effectiveness of antenatal betamethasone. There were no differences found in neonatal biometric measures or need for mechanical ventilation for respiratory distress syndrome. Concerns that added maternal plasma volume in multiple gestations could lessen the neonatal benefits of antenatal betamethasone were not substantiated. A small sample size confers the possibility of beta-error, and the retrospective nature of this investigation invites the possibility of sampling bias leading to the differences in major adverse outcome measures.

650

Test Characteristics of a Neonatal Brachial Plexus Injury Prevention Score. John E Deaver, Wayne R Cohen.* *Obstetrics and Gynecology, Jamaica Hospital Medical Center, Jamaica, NY, USA.*

OBJECTIVE: To devise a clinically useful risk score for neonatal brachial plexus injury (BPI) using only variables available to obstetricians before delivery. The goal was a risk score that could be evaluated prospectively to determine whether a meaningful number of these injuries could be prevented without excessively increasing the cesarean section rate.

METHODS: A consecutive series of 45 cases of neonatal BPI and 90 controls was studied. Multiple logistic regression coefficients were obtained for maternal characteristics and intrapartum variables that influenced the odds of BPI. To identify clinically relevant categories, continuous variables were divided into quartiles. The regression coefficients were used to devise a risk score for BPI. The score's receiver-operator characteristics (ROC) curve was plotted.

RESULTS: Pertinent variables in the risk score were clinically estimated fetal weight (EFW), maternal term body mass index (BMI), pregnancy weight gain, gestational diabetes (GDM), black race, and durations of the deceleration phase and the second stage of labor. The odds ratios for 4th quartiles of EFW (≥3600 grams), maternal BMI (≥33.3 kg/m²), and pregnancy weight gain (≥18.2 kg) were 4.8 (p<0.001), 2.6 (p=0.07), and 2.8 (p< 0.05), respectively, and for GDM and black race were 2.3 (p= 0.23) and 3.3 (p<0.05). Long second stage labor increased the odds of BPI by 20.1 times (p<0.001) when preceded by a long deceleration phase and by only 2.7 times (p=0.13) otherwise. The risk score was calculated as $1 / (1 + e^{-x})$ where x was calculated from the regression coefficients of the pertinent variables listed above. The area under the ROC curve was 0.82. A risk score of 0.72 had sensitivity and specificity 36% and 99% respectively. The positive predictive value at this score cutoff was 7.3%, assuming a baseline BPI prevalence of 0.25%, the midpoint of literature-reported prevalence range.

CONCLUSION: If the risk score were applied to a population of 10,000 births that would otherwise have delivered vaginally with a BPI prevalence of 0.25%, 119 cesarean sections would be necessary to prevent 9 of 25 BPI cases. In other words, a 1.2% rise in the cesarean rate would prevent 36% of BPI cases. This is the first attempt to model multiple factors known before delivery and to examine the interaction of labor components in relation to BPI. We will next determine whether these test characteristics are reproducible when the model is applied to a novel dataset.

651

Incidence of Umbilical Artery pH < 7.0 in Elective Cesarean Section at Term in Women without Medical or Known Fetal Complications. David J Berke, Nicole M Adair, Guillermo J Valenzuela.* *Women's Health, Arrowhead Regional Medical Center, Colton, CA, USA.*

Intrauterine hypoxia has been associated with long-term fetal sequelae. Its detection via nonreassuring electronic fetal monitoring during labor is often postulated to be the cause of any newborn damage found at birth or thereafter. Although there are many reports discussing umbilical cord pH values on newborns during labor and under different clinical conditions, we decided to explore the incidence of newborn pH <7.0 on a group of patients undergoing elective cesarean sections at term, without maternal complications, without fetal malformations detected prior to delivery, and at a gestational age >38 weeks.

Material and Methods: Under a protocol approved by the IRB, records of all elective cesarean sections between 4/2004 and 6/2006 were reviewed. Information regarding maternal age, gravidity, parity, antenatal testing on

admission (NST), presence of uterine contractions, and umbilical cord gases were obtained. Patients with any medical complications or significant uterine contractions (including cervical change) were excluded. Newborn records were reviewed for any complications.

Results: A total of 333 records qualified by meeting all of the criteria. The average umbilical cord pH was 7.24 ± 0.08, base deficit of 3.9 ± 2.8. Six patients had pH < 7.0and all of these had base deficit =<12. All of them had 1 and 5 minute Apgars ≥ 7; one newborn was admitted to NICU (transient tachypnea). In the group with pH >7, there were 16 admissions to NICU, one with seizures.

Conclusions: About 2% of normal pregnant patients, without evidence of maternal complications, carry newborns that are acidotic by standard definition. However, all of these infants had normal Apgar scores and were discharged home without evidence of any deleterious effect. We speculate that there are factors other than just hypoxia that are necessary to produce fetal damage.

652

Treatment with 17-Alpha Hydroxyprogesterone Caproate (17-OHPC) Reduces NICU Length of Stay (LOS) by Improvement in Gestational Age at Delivery. Jennifer G Smith. (SPON: Paul Meis). *NICHD MFMU Network, Bethesda, MD, USA.*

Objective: To determine if improvements in neonatal outcome and NICU LOS in infants born to high risk mothers treated with 17-OHPC were independent of gestational age at delivery.

Materials and Methods: This is a secondary analysis of data from a multicenter trial of 17-OHPC for prevention of recurrent spontaneous preterm birth. Women with a history of a prior spontaneous preterm delivery were randomized in a 2:1 ratio between 16-20 weeks gestation to receive either weekly injections of 250 mg of 17-OHPC or placebo until delivery or 36 weeks gestation. NICU LOS and estimated cost of hospitalization was compared between groups. A composite measure of neonatal morbidity (1 or more of: RDS, BPD, IVH, ROP, NEC, patent ductus requiring closure, death) was compared between groups. Statistical analysis was performed by Chi-square and Wilcoxon tests. Adjusted statistical analysis was performed with logistic regression and Cox proportional hazard survival regression analysis.

Results: A total of 446 live births occurred during the study period. NICU admissions tended to be greater in the placebo group, but this difference did not reach significance (RR=0.76, 95% CI 0.58-1.01). Infants born to 17-OHPC treated mothers had approximately one half the risk of neonatal morbidity compared to those whose mothers received placebo (RR 0.59, 95 % CI 0.37-0.93). NICU LOS was significantly longer for the group of infants whose mothers were treated with placebo (p=0.02). After adjusting for gestational age at delivery, there were no significant differences in NICU LOS or composite neonatal morbidity between treated groups.

Neonatal Outcomes

	Admissions to NICU %(n)	Composite Neonatal Morbidity % (n)	Median NICU LOS* (days)	Mean NICU LOS (days)
17-OHPC (n=295)	27.8 (82)	10.8 (32)	13.0	22.4
Placebo (n=151)	36.4 (55)	18.5 (28)	22.0	32.5

*infants who died in the NICU were assigned a maximal LOS

Conclusions: Administration of 17-OHPC to women with a history of prior preterm delivery decreases NICU LOS and neonatal morbidity. Later gestational age at delivery is responsible for these improvements in outcome. Based on an average daily NICU cost of $5042/d[1], this translates into a cost savings of $50,928 per infant discharged from the NICU.

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653

Comparison of Perinatal Outcomes in the Setting of Chorioamnionitis among Different Ethnic Groups. Natali Aziz, Allison Bryant, Susan H Tran, Yvonne W Cheng, Aaron B Caughey. (SPON: Julian T Parer). *Obstetrics, Gynecology and Reproductive Sciences, Unviversity of California, San Francisco, San Francisco, CA, USA.*

Objective: Ethnicity has been identified as a risk factor for chorioamnionitis. The purpose of this study was to compare perinatal outcomes among different ethnic groups in the setting of chorioamnionitis.

Study Design: A retrospective cohort study was conducted at a single university medical center of all term deliveries occurring from 1980 to 2001 with the diagnosis of chorioamnionitis. Patients of different ethnic backgrounds were compared. Obstetric outcomes examined included mode of delivery, perineal lacerations, maternal blood loss, transfusions, placental abruption, and endomyometritis. Neonatal outcomes, including Apgar scores, umbilical cord gases, meconeum, meconeum aspiration syndrome (MAS), hyperbilirubinemia, sepsis, tachypnea, respiratory distress syndrome (RDS), and admission to neonatal intensive care unit were also assessed. Dichotomous outcomes were compared using chi-square test. Multivariate logistic regression models were created to evaluate obstetric outcomes and complications controlling for potential confounding factors.

Results: 1915 patients diagnosed with chorioamnionitis had available ethnic identifiers: Caucasian 689 (36%), African American 296 (15%), Latina 238 (12%), and Asian 692 (36%). In multivariate analyses, ethnicity was associated with difference in endometritis, 3rd/4th degree laceration, Apgar < 7 at five minutes, meconeum, and hyperbilirubinemia. (Table 1)

Conclusions: In addition to ethnicity being a risk factor for development of chorioamnionitis, our study demonstrates that different ethnic groups are at risk for increased adverse perinatal outcomes in the setting of chorioamnionitis.

Ethnicity and Perinatal Outcomes in Setting of Chorioamnionitis

	Af Am, AOR (95% CI)	Latina, AOR (95% CI)	Asian, AOR (95% CI)
3rd /4th Degree Laceration	1.32 (0.61-2.87)	1.02 (0.52-2.01)	1.84 (1.20-2.82)
Endometritis	1.30 (0.52-3.27)	1.68 (0.76-3.67)	2.22 (1.24-3.96)
5-min Apgar < 7	2.85 (1.12-7.23)	2.38 (1.08-5.22)	0.79 (0.40-1.55)
Meconeum	1.88 (1.21-2.94)	1.03 (0.69-1.55)	1.03 (0.77-1.38)

654

Congenital Cystic Adenomatoid Malformation: Prenatal Sonographic Findings and the Prediction of Postnatal Outcome. Amy H Picklesimer, Jennifer Chancellor, Nancy DeMaria, Honor M Wolfe. (SPON: Kim Ann Boggess). *Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

Objective: To identify prenatal sonographic predictors of postnatal outcome in fetuses with congenital cystic adenomatous malformation (CCAM).

Study design: Retrospective review of fetal CCAM at the University of North Carolina in Chapel Hill from 1999 until 2006. CCAM lesions were classified as microcystic, or macrocystic/mixed. Ultrasound parameters included type of CCAM, mediastinal shift and ultrasonographic "disappearance" of the lesion. Outcome data included need for NICU admission and need for surgery. Comparisons were made using Chi Square and Fisher's Exact Test.

Results: Nineteen CCAMs were identified at a mean gestational age of 24 0/7 weeks, ±6 weeks. There was one fetus with hydrops (5%), and this was the only neonatal death (5%). Postnatal imaging confirming the presence of a lung mass in all cases. Outcomes are summarized in Table 1. NICU admission was more likely for infants with macrocystic/mixed type than microcystic lesions (p=.06), as well as for those with mediastinal shift (p=.14). Surgical therapy was required more often for infants with macrocystic/mixed type (p=.01), as well as for fetuses demonstrating a mediastinal shift (p=.05). Classification of lesion as microcystic or macrocystic/mixed type did not predict likelihood of disappearance (p=.7).

Conclusion: CCAM remain a significant source of neonatal morbidity and mortality. Because of high rates of NICU admission, these infants benefit from delivery in close proximity to a Level III NICU. The prognosis appears to be better for lesions with a microcystic appearance, and for those that do not cause a mediastinal shift. The phenomenon of ultrasonographic "disappearance" was a common finding in this series, but seems to be associated with technological limitations of ultrasound rather than with actual resolution of the CCAM lesion.

Ultrasound Parameters and Postnatal Outcomes

	Microcystic n=11	Macrocystic/Mixed n=8	Total n=19
Mediastinal Shift	6 (55%)	7 (88%)	13 (68%)
Prenatal Resolution	5 (45%)	3 (38%)	8 (42%)
NICU Admission	4 (36%)	7 (88%)	11 (58%)
Surgical Treatment	7 (64%)	8 (100%)	15 (79%)

655

Perinatal Outcomes in Active Phase Arrest and Vaginal Delivery. Dana E Myers, Yvonne W Cheng, Brian L Shaffer, Anjali J Kaimal, Katherine Y Bianco, Caughey B Aaron. (SPON: Linda C Giudice). *Obstretrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: To determine the effect of active phase arrest on perinatal outcomes in women who subsequently deliver vaginally.

Study Design: This is a retrospective cohort study of women with term, singleton, cephalic neonates delivered vaginally at a single institution. Those diagnosed with active phase arrest (APA), no evidence of cervical change for two or more hours with adequate contractions, were compared to other women with vaginal deliveries. The relationship between active phase arrest and perinatal outcomes was evaluated using the chi-square test and multivariate logistic regression models.

Results: Of the 335 women with APA, 95 (28%) of these underwent an operative vaginal delivery as compared to the 17% (p<0.001) of women who did not have active phase arrest. In univariate comparisons, women with APA were more likely to experience chorioamnionitis, third or fourth degree perineal lacerations, and postpartum hemorrhage. When controlling for potential confounders, the increase in chorioamnionitis and postpartum hemorrhage remained statistically significant. However, there was no longer an appreciable difference in severe perineal lacerations. The rates of neonatal morbidity were low in both groups, with no statistically significant differences.
Conclusion: Women with APA who experience vaginal births have increased rates of postpartum hemorrhage and chorioamnionitis. The effect of APA on perineal lacerations appears to be due to confounding. These findings can be utilized to counsel women considering expectant management in the setting of APA in labor.

Table 1: Maternal Outcomes in Women with Active Phase Arrest

	No APA	APA	p value	AOR (95% CI)
Chorio	7.6%	18.2%	< 0.001	2.08 (1.52-2.85)
Peri 3 or 4	8.9%	15.5%	< 0.001	1.20 (0.85-1.68)
PPH	17.3%	26.2%	< 0.001	1.39 (1.06-1.82)

656

Comparison of Perinatal Outcomes in Patients with and without Epidural Anesthesia in the Setting of Chorioamnionitis. Natali Aziz, Susan H Tran, Yvonne W Cheng, Aaron B Caughey. (SPON: Julian T Parer). *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: It is unclear whether chorioamnionitis diagnosed in the setting of epidural is associated with worse perinatal outcomes, as maternal fever due to an epidural may alone be misdiagnosed as chorioamnionitis. The purpose of this study was to compare perinatal outcomes between women with epidural anesthesia and those without in the setting of chorioamnionitis.

Study Design: A retrospective cohort study was conducted at a single university medical center of all term deliveries occurring from 1980 to 2001 with the diagnosis of chorioamnionitis. Patients who delivered with epidural anesthesia were compared to patients who delivered without epidural anesthesia. Obstetric and neonatal outcomes were examined. Dichotomous outcomes were compared using chi-square test. Multivariate logistic regression models were created to evaluate obstetric outcomes and complications controlling for potential confounding factors.

Results: 2050 patients were diagnosed with chorioamnionitis. Of those, 1928 (94%) had epidural anesthesia. Patients with the diagnosis of chorioamnionitis who had epidural anesthesia were only more likely to have a cesarean delivery and PPH, and less likely to have placental abruption compared to those women who did not have epidural anesthesia in univariate and multivariate analyses. However, in univariate and multivariate analyses assessing neonatal outcomes in the setting of chorioamnionitis, epidural anesthesia was not associated with more adverse neonatal outcomes.

Conclusions: Overall, epidural anesthesia in the setting of chorioamnionitis was not associated with more significant adverse maternal and neonatal outcomes. Although we initially postulated that chorioamnionitis in the setting of an epidural may be associated with less adverse perinatal outcomes, our study does not support this hypothesis.

Epidural and Adverse Perinatal Outcomes in Setting of Chorioamnionitis

Outcome	Epidural vs. No Epidural	p-value	Adjusted OR (95% CI)
Cesarean	41.5% vs. 20.5%	<0.001	3.20 (1.73-5.92)
PPH	43.5% vs. 27.7%	0.001	1.64 (1.02-2.63)
Abruption	0.6% vs. 3.3%	0.001	0.24 (0.06-0.98)

657

Maternal Morbidity Associated with Postpartum Anticoagulant Exposure. Wendy Kinzler,* Antoinette Ham, Cande Ananth, William Scorza, Anthony Vintzileos.* *Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA.*

Objective: To compare postpartum complications between women receiving both antepartum/postpartum anticoagulants and women unexposed to anticoagulants.

Methods: This was a retrospective chart review of women delivered at our institution between 01/2002 and 06/2006. Women who received both antepartum and postpartum anticoagulants were identified from discharge diagnosis codes. Exposed and unexposed women were matched in a 1:2 fashion by mode of delivery using a random function generator. Prenatal and hospital records were reviewed. Paired t-test was used with P<0.05 considered to be statistically significant.

Results: A total of 14 exposed vaginal deliveries and 14 exposed cesarean deliveries were identified. Maternal thrombophilia (acquired and inherited) was the most common indication for anticoagulant use (64.3%, n=18). Exposed women had longer lengths of stay than unexposed for both vaginal (4.3 ± 4.6 versus 2.5 ± 0.9 days, P=0.05) and cesarean (5.4 ± 3.4 versus 3.7 ± 0.6 days, P=0.01) deliveries. There were no differences in postpartum hemoglobin levels. Postpartum complications (fever, wound complications, hematoma, transfusion) were found in 5 women in the exposed group (17.9%) compared with 5 women in the unexposed group (8.9%), P = 0.23. A complication rate of 66.7% was found among the 3 women who received therapeutic anticoagulation compared with 12% in the women who received prophylactic anticoagulation. Mean time to restart anticoagulants postpartum in those with complications was 8.0 ± 8.0 hours compared with 19.2 ± 8.5 hours in those without complications (P = 0.01).

Conclusions: Restarting anticoagulants postpartum is associated with an 18% complication rate, with the majority being postpartum fevers. These complications are associated with earlier resumption of anticoagulants.

658

Racial and Ethnic Differences among Women with Severe Postpartum Hemorrhage. Michael J Paglia,[1] Monique V Chireau,[2] Betty Thames,[2] Margaret Jamison,[2] Chad Grotegut,[2] Andra James.[2] (SPON: Edward K Chien). *[1]Obstetrics and Gynecology, Brown Medical School, Providence, RI; [2]Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.*

OBJECTIVE: The purpose of this study was to examine racial and ethnic differences among women who experience severe postpartum hemorrhage (PPH).

STUDY DESIGN: After approval by the Duke Institutional Review Board, records with the ICD-9 code 666.12 for immediate PPH were reviewed for the 5 year period from January 1, 2000, to December 31, 2004. Severe PPH was defined as immediate PPH requiring blood products. Using chi-squares, the proportions of African American and Hispanic women who experienced severe PPH were compared with the proportion of white women who experienced severe PPH.

RESULTS: During this period there were 12,476 deliveries. 29 out of 4831 white women, 36 out of 3871 African American women and 36 out of 2709 Hispanic women experienced severe PPH. (The Hispanic women were primarily of Central American, usually Mexican, origin.) The odds of severe PPH were, by definition, 1.0 for white women, the reference group. Compared to white women, the odds ratio (OR) for African American women was 2.05, 95% confidence interval (CI) 1.17, 3.61, p < .01, and for Hispanic women was 2.94, CI 1.68, 5.18, p < .01. 52% of the white women with severe PPH, 64% of the African American women with severe PPH and 31% of the Hispanic women with severe PPH were parous.

CONCLUSION: African American women and Hispanic women of Central American origin appear to be at an increased risk of PPH. The underlying reasons for these differences deserve investigation and may involve obstetrical or hemostatic factors.

659

Puerperal Group A Streptococcal Sepsis: Characteristics of Cases Requiring Surgical Intervention. Erin Clark,[1] Larry Wright,[2] Janice Byrne,[1] Kjersti Aagaard,[1] Robert Silver.*[1] *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA; [2]Infectious Diseases, LDS Hospital, Salt Lake City, UT, USA.*

OBJECTIVES: Puerperal Group A Streptococcal (GAS) sepsis is a rare yet potentially fatal infection. The clinical severity of infection varies widely, from mild endometritis that resolves with antibiotics, to invasive GAS that necessitates intensive care and/or surgery. We hypothesized that clinical and biological markers may be used to predict cases that ultimately require surgical intervention. As an extension to this hypothesis, we aimed to determine the rate of GAS vaginal colonization and to assess whether asymptomatic colonization is associated with subsequent puerperal sepsis and need for surgical intervention.

METHODS: 41 cases of puerperal GAS sepsis were identified in two tertiary care centers in Utah from 1991-2006. Cases were stratified based on need for surgical intervention. Characteristics of women who did and did not undergo surgery were compared using Fisher's exact or Chi-square analyses. The rate of GAS incidentally detected by routine Group B Streptococcus (GBS) surveillance cultures was calculated.

RESULTS: 17 (41%) required surgery including hysterectomy (N=8) and debridement alone (N=9). There were no maternal deaths. Patients requiring surgery were more likely to present in septic shock (10 vs. 3, p=0.003); require ICU care (13 vs. 3, p<0.005), pressor support (7 vs. 2, p=0.021), and/or ventilatory support (11 vs. 2, p<0.005); have ARDS (10 vs. 3, p=0.003), renal insufficiency (7 vs. 1, p=0.005), coagulopathy (8 vs. 2, p=0.008), soft tissue infection (8 vs. 0, p<0.005), pleural effusions (11 vs. 1, p<0.005), acites (9 vs. 2, p=0.003), thrombocytopenia (5 vs. 0, p=0.008), bandemia (15 vs. 13, p=0.039), and/or leukopenia (4 vs. 0, p=0.024). The rate of GAS positive GBS surveillance cultures is 0.03% (48,828 samples). Only two cases (one required surgery) had evidence of GAS colonization prior to delivery (0.05%).
CONCLUSIONS: Puerperal GAS sepsis continues to be an uncommon but important cause of maternal morbidity. Septic shock, ICU care, pressor/ventilatory support, ARDS, renal insufficiency, coagulopathy, soft tissue infection, pleural effusions, acites, thrombocytopenia, bandemia, and leukopenia were associated with subsequent surgical intervention. The majority of patients with puerperal GAS sepsis had no evidence of antecedent colonization on routine vaginal/perineal GBS surveillance cultures.

660

The MSX1 Allelele 4 Homozygous Child Exposed to Periconceptional Smoking Is Sensitive To Develop a Nonsyndromal CLP. Marie-Jose H van den Boogaard,[1] Dominique de Costa,[1,2] Fan Liu,[2] Cock M van Duijn,[2] Dick Lindhout,[1] Regine PM Steegers-Theunissen.[2,3,4,5] (SPON: Eric AP Steegers). *[1]Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; [2]Epidemiology and Biostatistics; [3]Obstetrics and Gynecology; [4]Pediatric Cardiology; [5]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*
Background: The relationship between genes and nonsyndromic cleft lip and/or cleft palate (CLP) may be modified by intrauterine exposures. The MSX1 gene is highly expressed in tissues of the oral cavity during embryogenesis.
Objective: To investigate interactions between the CA-repeat in the MSX1 gene and four periconceptional lifestyle exposures in relation to the risk of CLP offspring.
Methods: In a Dutch case-control study on CLP, we examined CA repeats in the MSX1 gene and the interactions with the periconceptional exposures smoking, alcohol consumption, medication use and folic acid supplementation. We included 181 case and 132 control mothers, 155 case and 121 control fathers, and 176 case and 146 control children.The CA-repeat was determined by Polymerase Chain Reaction followed by fragment analysis. The exposures were obtained by questionnaires. Multivariate logistic regression analysis was applied and Odds ratios (OR) and 95% confidence intervals (CI) were calculated.
Results: The CA-repeats in allele 4 were most frequent in case- and control-triads without affecting CLP risk. In the periconception period case-mothers used significantly more medication (p≤ 0.01). Case-fathers smoked more than controls (p=0.05). Interactions were observed between allele 4 homozygosity of the child and materal and paternal smoking, OR: 2.7; 95%CI:1.1-6.5 and OR: 2.2; 95%CI:1.0-4.7, respectively. Mothers homozygous for allele 4 who did not use folic acid showed a 2.3 fold increased risk of CLP offspring (95% CI: 1.0-5.0). Maternal and paternal medication use and allele 4 homozygosity in the mother and father was associated with an increased CLP risk, OR:8.3; 95% CI: 2.4-28.7 and OR:3.3; 95% CI: 0.9-12.0. No significant interactions could be found between allele 4, parental alcohol use and CLP risk.
Conclusion: The MSX1 allele 4 homozygous child exposed to periconceptional smoking by both parents is sensitive to develop a CLP. Interaction between medication and MSX1 allele 4 homozygosity in the parents may suggest a function of MSX1 in the detoxification pathway.

661

Characteristics of Second Trimester Pregnancy Loss According to Gestational Age. Wendy Kinzler,* Kathleen DiBiase, Cande Ananth, Anthony Vintzileos.* *Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA.*
OBJECTIVE: Our objective was to determine differences in 2nd trimester pregnancy loss characteristics according to gestational age at time of loss.
STUDY DESIGN: Patients referred to our Pregnancy Loss Evaluation Service (PLES) from October 2003 to May 2006 were comprehensively evaluated, including a detailed medical history, appropriate laboratory testing, sonohysterography and review of placental pathology. Women with at least one pregnancy loss (spontaneous and fetal demise) between 14 and 23 weeks' gestation were included for review. Pregnancies were categorized as 14-16 weeks', 17-19 week' or 20-23 weeks' gestation at time of loss. Comparisons for each of these groups were made for the presence of vaginal bleeding, preterm rupture of membranes and placental histology.
RESULTS: 266 women were evaluated by the PLES and 30% (n=80) had at least one 2nd trimester PL, for a total of 120 fetal losses. Of these, 75% (n=90) were singletons and 67.8% (n=78) were spontaneous conceptions. Antepartum fetal demise prior to delivery was noted in 20.3% (n=24). Mean maternal age ± SD at loss was 31.5 ± 5.9 years and mean gestational age at loss ± SD was 18.8 ± 2.5 weeks. Table 1 shows the characteristics for each of the gestational age groups.
CONCLUSION: PROM is more common at 17-19 weeks' gestation compared with losses occurring in the earlier or later second trimester. Losses at 17-23 weeks' gestation are more likely to be associated with acute infection and chronic inflammatory lesions compared with losses at 14-16 weeks gestation. There is a trend towards more thrombotic lesions when losses occur in the early second trimester.

Pregnancy loss characteristics according to gestational age

	A: 14-16 wks (n=28)	B: 17-19 wks (n=43)	C: 20-23 wks (n=49)
	% (n)	% (n)	% (n)
Bleeding	46.4% (13)	67.4% (29)	67.3% (33)
PPROM	39.3% (11)*	60.5% (26)***	38.8% (19)
Acute infection	25.0% (7)*/**	55.8% (24)	57.1% (28)
Chronic inflammation	7.1% (2)*/**	34.9% (15)	28.6% (14)
Decidual lesions	46.4% (13)	41.9% (18)	55.1% (27)
Thrombotic lesions	17.9% (5)	11.6% (5)	6.1% (3)

*P<0.05 for A vs B,**P<0.05 for A vs C, ***P<0.05 for B vs C

662

Prediction of Pre-Eclampsia and Small-for-Gestational-Age Babies in High-Risk Pregnancies in the VIP (Vitamins in Preeclampsia) Trial. Paul T Seed,[1] Robyn A North,[2] Katrina K Poppe,[2] Mik Black,[2] Lucilla Poston.*[1] *[1]Maternal & Fetal Research Unit, Division of Reproduction & Endocrinology, King's College London, United Kingdom; [2]Dept Obstetrics and Gynaecology, School of Population Health, University of Auckland, New Zealand.*
Introduction
Previous pre-eclampsia, obesity and pre-existing disease carry an increased risk of preeclampsia and SGA deliveries. Currently we cannot accurately estimate the risk of preeclampsia or SGA in women with several risk factors. The VIP trial provided the opportunity to investigate prediction using sets of risk factors.[1]
Objective
To develop risk assessment tools for preeclampsia and SGA based on sets of clinical variables drawn from the VIP trial database.
Methods
1,687 women with singleton pregnancies, with obesity (BMI>30), chronic hypertension or previous preeclampsia were randomly divided into training (n= 1121) and validation (n=566) sets. Outcomes in the validations set were strictly concealed. Prediction tools were developed on the training set only using logistic regression, with forward variable selection.
The outcomes were preeclampsia, preeclampsia delivered < 34 weeks; SGA (< 10th customised centile), and severe SGA (SGA delivered < 34 weeks/ birthweight < 1st centile/perinatal death). Prediction tools were developed on the training set using logistic regression with forward variable selection.
Predictors used in the final models were: previous PE disease, and gestation, chronic hypertension, maternal age, smoking during pregnancy, parental ethnicity, use of folate and multivitamins, anti-hypertensive therapy, BMI, SBP and DBP. Other variables considered included creatinine levels pre-pregnancy, parity, marital status, highest academic qualification, employment, activity in last week, randomised treatment with antioxidants, use of aspirin, heparin and fragmin, and gestation at trial recruitment.
Results

Prediction of pregnancy complications using clinical data: performance

	PE	Early PE	SGA	Severe SGA
Prevalence %	14	3.7	24	8.8
Sensitivity %	53	43	49	54
Specificity %	72	90	65	67
PPV %	24	14	32	14
NPV %	91	98	80	94
Positive Likellihood ratio	1.92	4.09	1.40	1.65

Conclusions
Whilst not of adequate predictive power as a "stand-alone" test, the combination of these clinical risk factors with other predictive variables, e.g. biomarkers may prove a useful tool for identification of high risk women destined to develop preeclampsia or deliver SGA babies.
Reference
1 Poston L et al. *Lancet* 2006 367 pp 1145-11154.

663

Obstetrical Management of Severe Postpartum Hemorrhage. Michael J Paglia,[1] Chad Grotegut,[2] Betty Thames,[2] Andra James.[2] (SPON: Edward K Chien). *[1]Obstetrics and Gynecology, Brown Medical School – Women and Infants Hospital, Providence, RI; [2]Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.*

OBJECTIVE: The purpose of this study was to catalog interventions used to manage severe postpartum hemorrhage (PPH) at a single institution.

STUDY DESIGN: After approval by the Duke Institutional Review Board, records with the ICD-9 code 666.12 for immediate PPH were reviewed for the 5-year period from January 1, 2000, to December 31, 2004. Severe PPH was defined as immediate PPH requiring blood products. Interventions used in the management of severe PPH hemorrhage were noted

RESULTS: During this period there were 12,476 deliveries. 671 records had a diagnosis of immediate PPH and were reviewed. 108 met inclusion criteria for severe PPH. For initial management, all women received pitocin infusion per institutional protocol. In addition, 68/108 women received either methergine (n=51), prostaglandin F2α (n=45), misoprostol (n=18) or some combination of the 3. Misoprostol was used alone in 1 case, after methergine in 4 cases, after prostaglandin F2α in 3 cases and after both in 10 cases. Surgical interventions included curettage (n=16), B-Lynch suture (n=7), uterine or vaginal packing (n=4), uterine artery ligation (n=4), progressive uterine devascularization (n=1) and intrauterine balloon placement (n=3). 15/108 underwent cesarean hysterectomy. In 7/15 cases, hysterectomy was preceded by one or more interventions. Hysterectomy followed 3/16 curettages, 2/7 B-Lynch sutures, 1/4 instances of uterine or vaginal packing, 2/4 uterine artery ligations, 0/1 uterine devascularization procedures and 1/3 balloon placements. 1 of the 3 balloon placements was preceded by curettage and followed by packing and uterine artery embolization and 1 of the uterine artery ligations was followed by B-Lynch suture. No hysterectomy was necessary in either of these cases.

CONCLUSION: Uterotonics alone were successful in 70/108 (65%) of cases of severe PPH. Other interventions were required in 38/108 (35%) of cases. These interventions were successful in averting hysterectomy in 23/38 cases or 60% of the time, but hysterectomy was ultimately required in 14% of cases with severe PPH.

664

Factors Associated with Severe Postpartum Hemorrhage. Michael J Paglia,[1] Chad Grotegut,[2] Betty Thames,[2] Eleanor Rhee,[2] Andra James.[2] (SPON: Edward K Chien). *[1]Obstetrics and Gynecology, Brown Medical School – Women and Infants Hospital, Providence, RI; [2]Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.*

OBJECTIVE: The purpose of this study was to identify factors associated with severe postpartum hemorrhage (PPH) at a single institution.

STUDY DESIGN: After approval by the Duke Institutional Review Board, records with the ICD-9 code 666.12 for immediate PPH were reviewed for the 5-year period from January 1, 2000, to December 31, 2004. Severe PPH was defined as immediate PPH requiring blood products. Associated factors were identified.

RESULTS: During this period there were 12,476 deliveries. 671 records had a diagnosis of immediate PPH. 108/671 received blood products and met inclusion criteria. Of the 108, the mean age was 28 and the median parity was 1. Only 4 had a history of PPH. Only 3 were grandmultiparas (parity ≥ 5). There were 10 sets of twins and 1 set of triplets. 9/108 had placenta previa, 6/108 placental abruption, 7/108 accreta, 6/108 retained placenta and 1/108 uterine inversion. 30/108 had a diagnosis of preeclampsia, 4/108 had HELLP syndrome and 19/108 received magnesium sulfate. 14/108 had chorioamnionitis. 31/108 had undergone induction of labor and another 29/108 had been augmented with pitocin. 12/108 had been delivered by vaccuum extraction or forceps. Another 60/108 had undergone cesarean delivery. 7/108 had none of the preceding risk factors.

CONCLUSION: Only 36/108 (33%) of women had one or more of the following risk factors associated with severe PPH: history of PPH, grandmultiparity, multiple gestation, previa, abruption, accreta, retained placenta or uterine inversion. Using a broader list of risk factors that also included preeclampsia, magnesium sulfate, HELLP syndrome, chorioamnionitis, induction of labor, pitocin augmentation and operative delivery, 101/108 (94%) of women with severe PPH could be found to have one or more risk factors.

665

Abstract Withdrawn.

666

Use of Recombinant Factor VIIa for Massive Post Partum Hemorrhage. Nazli Hossain,[1] Tahir Shamsi,[2] Michael Paidas,[3] Nargis Soomro.[4] (SPON: Charles J Lockwood). *[1]Obstetrics, Gynecology and Reproductice Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Haematology, Bismillah Taqee Institute of Health Science, Karachi, Pakistan; [3]Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [4]Department of Obstetrics & Gynecology Unit 2, Dow University of Health Sciences, Karachi, Pakistan.*

OBJECTIVE: Massive postpartum hemorrhage (PPH) is defined as blood loss > 1500 ml. We hypothesized that patients with massive PPH may benefit from the use of activated recombinant Factor VII (rFVIIa), as a life saving measure.

MATERIALS & METHODS: All patients with PPH were admitted to the Surgical Intensive Care Unit of Civil Hospital, Karachi, Pakistan for evaluation and management. From March 2005 to September 2006, 18 patients who fulfilled the criteria of massive PPH and who received rFVIIA to regulate bleeding (study group) were compared with 18 who patients who fulfilled the criteria of massive PPH, but did not receive rFVIIa (control group). Physcian discretion, drug availability and drug cost influenced administration of rFVIIa during this time period. Main outcome measures were the amount of blood and blood products transfused, preservation of fertility, correction of disseminated intravascular coagulation, and maternal mortality risk.

RESULTS: Fourteen of 18 patients (78%) who received rFVIIa, survived, as compared to 8 of 18 women (44%) who also had massive PPH, but did not receive rFVIIa and died. Fertility was preserved in 13 of 18 (72%) study patientscompared to 8 of 18 (55%) patients in the control group.The mean number of transfusions in study was 10, compared to16 in the control group. The mean prothrombin times and activated partial thromboplastin times were significantly reduced in the study group following rFVIIa administration (32 sec to 12 sec; 52 to 29 sec, (*p value* .006 and .005 respectively). The mean ICU stay in the study group was 3.6 days, compared to 6.2 days in control group. No thromboembolic event or myocardial infarction was observed in the entire study population.

CONCLUSION: Activated recombinant factor VII can be a life saving drug in patients with massive PPH. Limitations of the drug include cost and availability. This case series represents the largest published experience with rFVIIa in a single institution. Additional studies are needed to evaluate the safety and efficacy of this promising, potentially life saving, measure in the setting of massive PPH.

667

The Use of Commercially Available Multivitamin Supplementation and Healthcare Resource Use. Annette Briley, Paul Seed, Andrew Shennan, Lucilla Poston.[*] *Division of Reproduction and Endocrinology, King's College London, London, Greater London, United Kingdom.*

Background: Pregnant women are advised to eat a healthy and varied diet, but apart from periconceptual supplementation of folic acid to prevent neural tube defects the decision whether or not to take a multivitamin supplementation is commonly left to the individual. As previously reported, 23% of high risk women participating in the Vitamins in Pre-eclampsia (VIP) trial (UK) were self medicating with commercially available multivitamin preparations at trial entry[1]. Supplementation did not reduce the incidence of pre-eclampsia or preterm birth, but there was a significant increase in birthweight and improved birthweight centiles in the babies of mothers who took supplements (in the placebo arm). As financial constraints become ever more pressing throughout the developed and developing world, simple measures to improve pregnancy outcome and limit resource use should be considered.

Methods: Data from the women in the placebo arm of the Vitamins in Pre-eclampsia trial were analysed, using the arithmetic mean to assess healthcare resource use in those who did and did not take commercially available micronutrient preparations.

Results

	Supplements (arithmetic mean)	No supplements (arithmetic mean)	Difference	95% CI	p
Pre-labour nights	3.0	2.0	1.1	0.3 - 1.9	0.009
Postnatal nights	4.0	3.6	0.6	-0.4 - 0.5	0.804
Total nights in hospital	7.0	6.0	1.1	0.081 - 2.20	0.035
Day Unit Attendences	2.4	2.4	0.2	-0.236 - 0.639	0.367
Days in NICU/SCBU	0.22	0.20	-0.325	-0.107 - 0.42	0.390
	% of women	**% of women**			
SVD	36	40	- 3	-10 - 4	0.393
Caesarean Section	55	46	8	1 - 15	0.030

Conclusions: Despite improved birthweights, pregnancy outcome resource use was increased in women who self medicated with commercially available micronutrient supplements. This may be indicative of increased risk status, or maternal anxiety. These data suggest that administration of multivitamin supplements does not reduce healthcare resource utilisation.

References: 1. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH. Vitamin C and Vitamin E in pregnant women at risk for pre-eclampsia (VIP Trial): randomised placebo-controlled trial. Lancet 2006;367: 1145-54

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668

Reduced Infant Birthweight Consequent upon Maternal Exposure to Severe Life Events. Ali S Khashan,[1] Roseanne McNamee,[3] Kathryn M Abel,[1] Louise C Kenny,[*2,5] Marianne G Pedersen,[4] Roger T Webb,[1] Preben B Mortensen,[4] Philip N Baker.[*2] *[1]Centre for Women's Mental Health Research, University of Manchester, United Kingdom; [2]Maternal and Fetal Health Research Center, University of Manchester, United Kingdom; [3]Biostatistics Group, University of Manchester, United Kingdom; [4]National Centre for Register-Based Research, Aarhus University, Denmark; [5]BUPA Ireland Research Center, Department of Obstetrics and Gynaecology, University College Cork, Ireland.*

Objectives: Stress has been associated with adverse pregnancy outcome. We sought to investigate the association between maternal exposure to severe life events and birthweight.

Methods:All women delivering singleton live births in Denmark between 1 January 1979 and 31 December 2002 (n=1.38million pregnancies) were linked to information on their spouses, parents, siblings and older children. Exposure was defined as death of a relative during pregnancy or in the six months prior to conception. Linear regression was used to examine the effect of exposure on birthweight. Models were controlled for gestational age, maternal age, infant sex, year of birth, parity and maternal medical history of diabetes, renal disease and hypertension.

Results: Birthweight was significantly decreased in the offspring of women exposed to the death of a relative during pregnancy or in the six months prior to conception. The highest decrease in birthweight was found in offspring of women who were exposed during the 2nd trimester (adjusted estimate β= -46gm [95% CI -61, -33]) (Table 1). Death of spouses, parents and siblings were also associated with reduced birthweight when analysed separately.

Conclusion: Mothers exposed to severe life events pre-conceptionally and during pregnancy appear to have babies with significantly lower birthweights. The potential mechanisms of stress-related effects on birthweight include changes in lifestyle due to the exposure and stress related disregulation of the hypothalamic pituitary adrenal axis during pregnancy.

Acknowledgments

This study was funded by Tommy's the Baby Charity.

Table1: Association between birthweight and maternal exposure to death of any relative

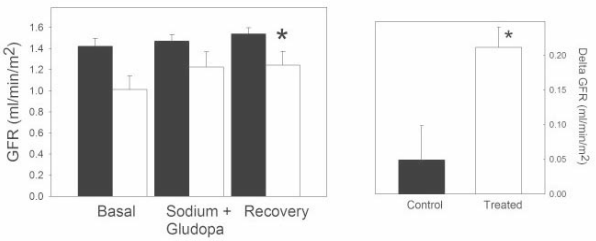
Exposure: death of any relative	Number of pregnancies	Adjusted estimate(β) and 95% CI
Unexposed	1024275	Reference
exposed before pregnancy	11235	-19(-28,-11)
exposed during 1st trimester	4240	-27(-41, -13)
exposed during 2nd trimester	4272	-47(-61, -33)
exposed during 3rd trimester	5657	-29(-41, -17)

669

Renal Function in Adult Sheep Exposed to Antenatal Glucocorticoids during Peak Nephrogenesis. James Perrot, Philip Deibel, Jie Zhang, Angela G Massmann, James C Rose,[*] Jorge P Figueroa.[*] *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

OBJECTIVE: In rats and sheep exposure to GC in the perinatal period induces hypertension in adult life. Intrarenal generation of dopamine is considered to be an important mechanism in the regulation of sodium excretion. Under basal conditions, 50% of sodium excretion is controlled by dopamine. Alterations of the dopamine system have been clearly associated with hypertension both in humans and in animal models. METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (CTR) 24 h apart at 80 days gestational age and allowed to deliver at term. At 1.5 yr of age, the offspring were chronically instrumented under general anesthesia to place intravascular catheters. In two separate experiments, the kidney's response to exogenous dopamine administered as the prodrug gludopa (18 µg/Kg/min) under basal conditions (1 hour) and following an acute sodium load (0.0275 mEq/Kg/min for 1 h) was compared to that of vehicle in BM and control sheep. Glomerular filtration rate (GFR) was measured as inulin clearance and effective renal plasma flow (ERPF) as PAH clearance. Dopamine D1 receptor (D1R) expression (mRNA and protein) was measured using Ribonuclease Protection Assay and western blotting. Data Mean±SEM were analyzed by ANOVA.

RESULTS: Under basal conditions, BM exposed sheep had lower GFR (left panel) but normal ERPF. Acute sodium loading did not change these parameters in either group. However, during gludopa infusion sodium loading significantly increased GFR (delta GFR right panel) only in the BM treated sheep. ERPF was not altered by gludopa infusion alone or during the simultaneous sodium loading. CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects on renal function. The increase response to dopamine stimulation during an acute sodium load suggests an alteration in the kidney's ability to regulate sodium. A decrease in dopamine natriuretic effects is associated with hypertension in humans and in animal models. HL 68728; HD P01 HD04784.



670

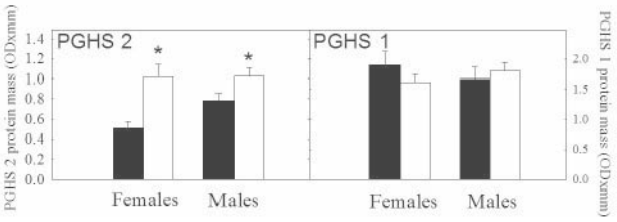
Antenatal Betamethasone Administration Selectively Increases Prostaglandin H Endoperoxide Synthase-2 Expression in Adult Sheep Kidney Medulla. Angela G Massmann, Jie Zhang, Jorge P Figueroa.[*] *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

OBJECTIVE: Prostaglandins are known to be important regulators of kidney growth and differentiation. In the adult kidney cortex PGHS-2 is an important modulator of renin production and secretion and in kidney medulla it regulates sodium handling. The aims of this study were to ascertain 1) if there are gender differences in renal PGHS expression and 2) if antenatal glucocorticoid administration has long term effects on PGHS-1 and PGHS-2 expression in adult sheep.

METHODS: Date-mated sheep were randomly assigned to receive a single course of GC [2 maternal IM doses of Betamethasone (0.17 mg/Kg with a maximum of 12 mg) or vehicle 24-h apart] at 80 days gestation. The offspring were euthanized at 0.8-1.5 yr of age to harvest the kidneys. Kidney medulla (KM) was obtained from 18 female (9 control, 9 GC) and 18 males (9 control, 9 GC) and immediately frozen in liquid nitrogen. PGHS-1 and PGHS-2, mRNA and protein were measured in KC and KM using Ribonuclease Protection Assay and Western Blotting. Data are expressed as Mean±SEM and were analyzed by ANOVA and t test.

RESULTS: No sex differences in either PGHS 1 or PGHS 2 expression were observed in the control group. The expression of PGHS-2 protein (left panel), but not that of PGHS-1 (right panel) was statistically significantly higher in KM of the GC exposed group (open bar). The increase was statistically significant for both males and females $p<0.05$ by t test.

CONCLUSION: In the adult kidney PGHS-2 participates in the regulation of sodium reabsorption. Low dietary salt decreases and high salt increases PGHS-2 expression in KM. Therefore this finding is consistent with an activation of salt reabsorption mechanisms which may explain the development of hypertension. HL 68728; HD P01 HD04784.



671

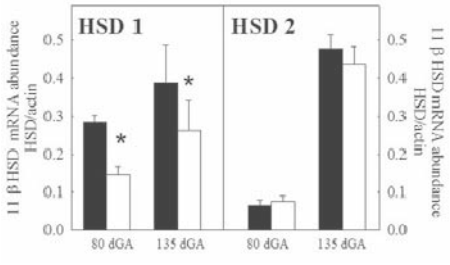
Antenatal Betamethasone Administration Decreases 11 ß Hydroxy Steroid Dehydrogenase (11 ß HSD) 1 Expression in Fetal Sheep Kidney. Jie Zhang, Angela G Massmann, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

OBJECTIVE: Fetal exposure to high levels of glucocorticoids (GC) is considered to be one of the mechanisms underlying Fetal Programming. In rats and sheep, exposure to GC in the perinatal period induces hypertension in adult life. However, no conclusive data exist on the potential detrimental effects of GC when administered at a gestational age equivalent to that of pregnant women threatened with premature labor. The two isoforms (1 and 2) of the enzyme 11 β-HSD catalyze the interconversion of active cortisol and inactive cortisone. 11 β-HSD 2 is the predominant isoform in kidney where is thought to regulate cortisol exposure in cells expressing mineralocorticoid receptors. The aim of this study was to compare expression levels of 11 β-HSD 1 and 2 in kidney cortex (KC) and medulla (KM) in sheep exposed antenatally to a single course of GC at 0.55 of gestation.

METHODS: Date-mated sheep were randomly assigned to receive a single course of GC [2 maternal IM doses of Betamethasone (0.17 mg/Kg with a maximum of 12 mg) or vehicle 24-h apart] at 80 days gestation. Fetuses were delivered by C-section at either 80 or 135 days gestational age (dGA). KC and KM were obtained from fetuses and adults (0.8-1.5 y). 11 β-HSD 1 and 2 mRNA were measured by Ribonuclease Protection Assay. Data are expressed as Mean±SEM and were analyzed by ANOVA and t test.

RESULTS: Relative to actin mRNA, 11 β-HSD 1 mRNA levels were significantly higher than HSD 2 in KC and significantly decreased in KC and KM of GC-exposed fetuses at 80 and 135 dGA. However, in the adult 11 β-HSD 1 expression was not different. In contrast, antenatal Beta did not alter 11 β-HSD 2 expression in either KC or KM at any of the ages studied.

CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has acute and long-term effects on the kidney's ability to metabolize GC. The decrease in 11 β-HSD 1 expression during development suggests that it may play a role in the alterations in renal development known to exist following antenatal GC administration. HL68728, P01 HD04784.



672

Acute Effects of Betamethasone (BM) on Cortical Brain Function of the Ovine Fetus Depend on the Number of Treatment Courses. Christian Weiss,[1] Martin G Frasch,[1] Thomas Muller,[2] Harald Schubert,[2] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller University, Jena, Germany; [2]Inst. of Lab Animal Sciences, Friedrich Schiller University, Jena, Germany.*

Cortical acoustic evoked potentials (cAEP) are a measure of complex cortical brain function. Using fetal magnetencephalography, we have shown that the latency of cAEP component p2 is delayed in the human fetus 24h after BM injection (J Soc Gyn Inv 11,2004:69). Similarly, BM acutely alters electrocortical activity in fetal sheep (J Physiol 531,2001:535).

Objectives: To examine the time course of the BM effects on the cAEP and the effect of repeated treatment.

Methods: Pregnant ewes carrying chronically instrumented fetuses received 3 courses of 2x110µg/kg BM i.m. 24h apart (n=9) corresponding to 2x8mg BM to a 70kg pregnant woman or an equal volume saline (n=6) at 105, 112 and 119 dGA (days gestation, term 150 days). cAEP were evoked before and 4 and 24h after each BM injection using a tone of 500 Hz, 100 dB SPL and 50 ms duration with randomly chosen inter-stimuli intervals of 0.8–1.2sec applied at the maternal abdominal wall for 5 min. cAEP were recorded from the Cz position at the fetal skull and the components P1, N1, P2 and N2 were detected.

Results: At 105 dGA, BM treatment induced a latency increase of the P2 and N2 24h after the 2nd injection ($p<0.05$) but did not alter amplitudes. At 112 dGA, this delay already occurred 24h after the 1st injection in the the P2 and N2 (Fig. 1). In addition, the N1 was delayed 24h after the 2nd injection ($p<0.05$, Fig. 1). In parallel, amplitudes of the p2 and n2 decreased 4h after the 1st injection ($p<0.05$). At 118 dGA, BM did not induce a latency delay. Rather the latencies of all components were decreased 4h after the 1st and 2nd BM injection (in tendency or $p<0.05$). Amplitudes were not altered.

Conclusion: BM delayed the cAEP components during the 1st BM course as in the human fetus. This effect occurred earlier and was more extended during the 2nd course of BM. Disappearance of the effect during the 3rd BM course suggests programming of glucocorticoid resistance.

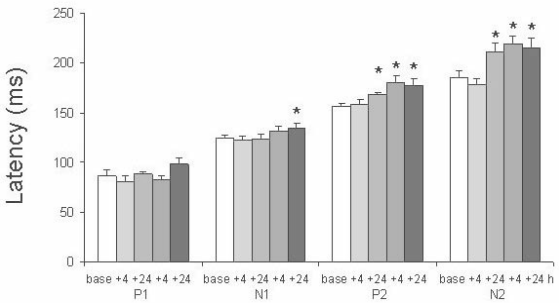


Fig. 1 BM effect on cAEP at 112 dGA. Mean+SEM, *$p<0.05$ *vs.* baseline.

673

Development of Cortical Acoustic Evoked Potentials (cAEP) in Fetal Sheep – Effects of Betamethasone (BM). Christian Weiss,[1] Martin Frasch,[1] Thomas Muller,[2] Harald Schubert,[2] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller Univ, Jena, Germany; [2]Inst. of Lab Animal Sciences, Friedrich Schiller Univ, Jena, Germany.*

BM at the dose used clinically to induce fetal lung maturation accelerates maturation of REM sleep ECoG (JSocGynInv 13,2006,731). Using fetal magnetencephalography we have shown that BM delays the latency of cAEP in the human fetus acutely reflecting alteration of cortical brain function (JSocGynInv 11,2004,69).

Objective: To examine if BM effects on cAEP persist. Therefore, development of cAEP during late gestation was monitored and chronic BM effects were examined.

Methods: Pregnant ewes carrying chronically instrumented fetuses received 3 courses of BM (2x110 µg/kg i.m. 24h apart corresponding to 2x8 mg BM to a 70 kg pregnant woman, n=9) or an equal volume saline (n=6) at 105, 112 and 119 dGA (days gestation, term 150 days). cAEP were induced before each BM course and six days after the last course using a tone of 500 Hz, 100 dB SPL and 50ms duration with randomly chosen inter-stimuli intervals of 0.8–1.2sec applied at the maternal abdominal wall for 5 min. cAEP were recorded from the Cz position at the fetal skull and the components P1, N1, P2 and N2 were detected.

Results: cAEP were detected in 7/9 BM and 5/6 saline treated fetuses at 105 dGA and later on in all fetuses. In controls, latency of all components decreased continuously until 125 dGA ($p<0.05$, Fig. 1). Amplitudes of the N1 and P2 started to increase at 125 dGA ($p<0.05$). Increase of the amplitudes was not affected by BM but developmental decrease of latencies was delayed. The P2 and N2 did not and the P1 and N1 started to decrease at 125 dGA ($p<0.05$, Fig. 1). Compared to controls, latency of all components was delayed at 125 dGA ($p<0.05$, Fig. 1).

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Conclusions: We detected cAEP in the sheep fetus for the first time and traced their development that is determined by myelination and maturation of neuronal circuits. Development of cAEP and, thus, of cortical brain function is affected by BM.

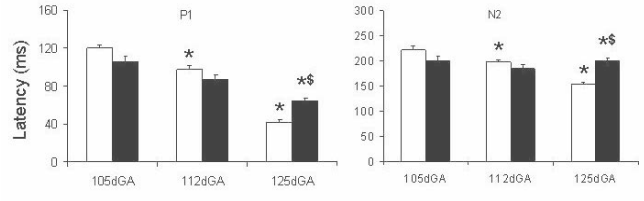


Fig. 1 Development of the latencies of the P1 and N2 in saline (white) and BM treated fetuses (black). Mean+SEM, *p<0.05 compared to 105 dGA, $p<0.05 compared to BM.

674

Effects of Antenatal Glucocorticoids on Functional Recovery of Ovine Cortical Brain Function after Asphyxia. Christian Weiss,[1] Thomas Muller,[2] Martin G Frasch,[1] Harald Schubert,[2] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller University, Jena, Germany; [2]Inst. of Lab Animal Sciences, Friedrich Schiller University, Jena.*

Fetal asphyxia for example induced by umbilical cord occlusion (UCO) commonly occurs during premature labor (Vannucci, Neurologist 1,1995:35). Mothers of these babies had frequently received betamethasone (BM) to induce fetal lung maturation.

Objective: To examine if BM affects the functional outcome of the cerebral cortex after asphyxia.

Methods: Four UCO of 4 min duration 26 min apart were induced in 16 chronically instrumented fetal sheep at 125 days gestation (term 150 days). Saline (n=8) or BM (3.3 mg/ml/h, n=8) was infused directly to the fetus over 48h beginning 24h prior to UCO. Cortical acoustic evoked potentials (cAEP) that are a measure of complex cortical brain function were induced immediately before, 4h and 24h after onset of BM infusion and 6h and 24h after the last UCO using a tone of 500 Hz, 100 dB sound pressure level and 50 ms duration with randomly chosen inter-stimuli intervals of 0.8–1.2sec applied at the maternal abdominal wall for 5 min. cAEP were recorded from the Cz position at the fetal skull. cAEP were averaged from 100ms prior to 500ms after the stimulus and the components P1, N1, and N2 were detected.

Results: BM induced a transient delay of all cAEP components 4h after start of infusion that has normalized at 24h, i.e. immediately before UCO. Amplitudes were not altered. In controls, UCO induced a delay of all cAEP components 6h after UCO and all but the P1 24h after UCO (p<0.05, Fig.1), i.e. only the P1 recovered within 24 h. During BM treatment, the latency increase of the P1 was delayed and of the N1 less pronounced (p<0.05, Fig. 1). UCO did not alter the cAEP amplitudes in both groups.

Conclusion: BM modulated recovery of cAEP from UCO.

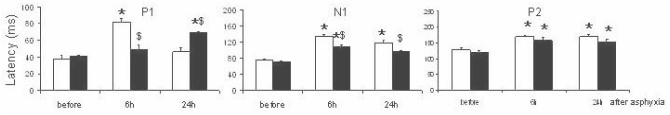


Fig.1 Latency of the cAEP after UCO in control (white) and BM treated fetuses (black). Mean+SEM, *p<0.05 compared to before UCO, $p<0.05 compared to BM.

675

Effects of Antenatal Glucocorticoids on Cerebral Protein Synthesis in the Preterm Ovine Fetus. Jeremy McCallum,[1] Norman Smith,[2] Matthias Schwab,*[3] Peter Nathanielsz,*[4] Bryan Richardson.*[1] *[1]OB/GYN, Univ. Western Ontario, London, ON, Canada; [2]Clinical Biochem, Univ. Western Ontario, London, ON, Canada; [3]Dept. Neurology, Friedrich Schiller University, Jena, Germany; [4]Center for Pregnancy and Newborn Res, Univ. Texas, San Antonio, TX, USA.*

Objective: While antenatal glucocorticoids have well known benefits for infants born preterm with enhanced pulmonary maturation, adverse effects on brain development have been reported in several animal studies. We have therefore determined the effects of antenatal glucocorticoids administered at doses used clinically on cerebral protein synthesis during brain development using ^{13}C-leucine tracer methodology.

Methods: Chronically instrumented pregnant sheep at 0.85 gestation received two IM injections of betamethasone at 170 µg/kg maternal weight or saline 24 hours apart together with a continuous infusion of ^{13}C-leucine to the fetus. Measurements were obtained for fetal plasma leucine enrichment (MPE_P) at steady-state and brain tissue intracellular free (MPE_{IF}) and protein bound (MPE_{PB}) leucine enrichment at necropsy, followed by the determination of cerebral fractional synthetic (FSR) and fetal leucine disposal rates (DR_{leu}). A coefficient of variation (CV) was determined for plasma and tissue enrichment measurements to assess the methodological variance using ^{13}C-leucine tracer technology.

Results: The cerebral FSR averaged 112 and 35 %/day when using the MPE_{IF} and MPE_P values for the precursor pool measurements, respectively, providing for maximal and minimal values, and with no differences between cortical and cerebellar tissues, nor between the control and the betamethasone animals. The DR_{leu} for both groups averaged 10.2 µmol/min/kg, with no observed changes as a result of glucocorticoid treatment. The CV for the MPE_P values averaged 2% while that for the MPE_{IF} and MPE_P values averaged 10% with an inverse relationship between the ^{13}C-leucine enrichment values and the CV values.

Conclusions: While cerebral FSR values for the preterm ovine fetus are high and indicate high rates of protein synthesis and degradation, we found no evidence that these are altered by betamethasone as used clinically and thereby do not account for the reported structural alterations in the brain following such therapy. We also found no effect of glucocorticoids on DR_{leu} as a measure of the rate at which leucine is used for protein synthesis and oxidation by the fetal organism, as well as loss across the placenta.

676

Prenatal Betamethasone (BM) Treatment at Clinical Doses Perturbs Myelination in the Fetal Sheep Brain Dependant on Gestational Age and Treatment Course. Iwa Antonow-Schlorke,[1] Alexandra Helgert,[1] Thomas Muller,[2] Harald Schubert,[2] Otto W Witte,[1] Peter W Nathanielsz,*[3] Matthias Schwab.*[1] *[1]Dept. of Neurology, Friedrich Schiller Univ, Jena, Germany; [2]Inst. of Lab Animal Sciences, Friedrich Schiller Univ, Jena, Germany; [3]Center for Pregnancy and Newborn Research, University of Texas, San Antonio, TX, USA.*

BM is routinely used to accelerate fetal lung maturation when preterm delivery is threatened. Four courses of BM at a dose higher than that used clinically delays myelination of the corpus callosum (Huang, Int J Dev Neurosci 2001). However, acute effects are not known. In addition, only single course of BM is recommended currently.

Aim: To show acute and chronic effects of BM at the dose used clinically on the expression of myelin basic protein (MBP) depending on the gestational age in fetal sheep.

Methods: To show acute BM effects, one course of BM (2x110 µg/ kg maternal body weight 24 apart (corresponding to 2x12 mg BM to a 70 kg pregnant woman) was administered to the ewe i.m. at either 80 (n=6), 95 (n=6), 110 (n=3) and 128 (n=6) days of gestation (dGA, term 150 days). Fetal brains were harvested 24h after the second BM injection. To show chronic effects, ewes received one course of BM at 110 (n=5) or two courses at 106 and 113 dGA (n=5) and fetuses were allowed to survive until 130 dGA. Control ewes received an equal volume of saline. Brains were fixed in paraformaldehyde and embedded in paraffin. The immunoreactivity (IR) of MBP was estimated by light microscopy and quantified using an image analysis system in the deep white matter of the fetal forebrain.

Results: MBP IR was detected first at 95 dGA indicating the onset of myelination. MBP IR increased continuously with gestation. BM acutely reduced MBP IR by 35.7 % (p<0.05) and 23.6 % (p<0.01) in the fetal brain at 95 and 110 dGA, respectively. This effect was reversed 20 days after a single but not double course of BM treatment (decrease of MBP IR by 42.8 %, p<0.01). In contrast to the effects at 95 and 110 dGA, BM did not affect MBP IR acutely at 130 dGA.

Conclusions: The results indicate a time window of MBP susceptibility for BM during early myelination. This is possibly due to a BM effect on oligodendrocytes that express MBP. The gestational age and stage of myelination vulnerable to glucocorticoids is comparable to that when BM is administered in human pregnancy. Disturbances of MBP seem to be reversible but might sustain after repeated BM treatment at the dose used clinically.

677

Plasma and Kidney Renin Concentration in Sheep after Prenatal Betamethasone Exposure. Lucia Kantorowicz, Nancy K Valego, Jorge P Figueroa,* James C Rose.* *Ob/Gyn, Wake Forest University Medical School, Winston-Salem, NC, USA.*

Objective

Antenatal glucocorticoid treatment is recommended in the management of preterm labour for the prevention of neonatal respiratory distress syndrome. Glucocorticoids are known to affect multiple organ systems. It has been proposed that fetal exposure to betamethasone (β) affects the renin-angiotensin system and thus might play a role in the development of hypertension. Our primary purpose in this study was to examine the effects of betamethasone exposure on prorenin (PRC) and active (ARC) renin concentration in plasma and kidney cortex from male sheep at 6 and 18 months of age.

Methods

Pregnant sheep were randomized to receive 2 doses of 0.17 mg/kg betamethasone or vehicle, given 24 hours apart at 80 and 81 days of gestation. The gestations continued until term. From 9 male offspring, at 6 months of age plasma samples were obtained, and at 18 months plasma and kidney were collected at necropsy. ARC and total renin concentration in plasma and homogenized kidney cortex were measured by RIA of angiotensin I generated by incubation with excess substrate. PRC is the difference between total and active in any sample.

Renal cortex was homogenized on ice in saline. Homogenates were centrifuged and frozen at -80 °C and diluted for RIA. Data are expressed as mean ± SEM and were analyzed by *t* test.

Results

In the *plasma,* at *6 months* of age, PRC was significantly lower in the β group than in the untreated group (4.63 ± 0.640 vs. 7.09 ± 0.835; p=0.034). At that age, ARC was similar in both groups. However, ARC was a significantly greater percent of the total plasma renin concentration in the betamethasone group (31.93 ± 4.093 vs. 18.57 ± 2.793; p=0.015).

In the *plasma,* at *18 months*, there was no difference in PRC, ARC or percent ARC between β and vehicle-treated groups, but there was an age-related decrease in ARC and PRC.

In the *homogenized kidneys*, at *18 months* of age, active and prorenin plasma concentration, and the percent active renin were similar in control and treated groups.

Conclusions

Our data suggest that prenatal exposure to betamethasone may alter the processing and the secretion of renin in the offspring at 6 months and this effect seems to disappear at 18 months. There is an age related decline in circulating renin concentration between 6 months and 18 months of age.

Supported by grants HL 68727, HD 17644 and HD 47584.

678

Bone Metabolism in Fetuses of Pregnant Women Exposed to Single and Multiple Courses of Corticosteroids. Linda Fonseca, Susan Ramin.* *for the NICHD MFMU Network, Bethesda, MD, USA.*

OBJECTIVE: A single course of antenatal dexamethasone has been associated with suppression of fetal collagen synthesis. The effect of repeated doses of antenatal corticosteroids on fetal bone metabolism is unknown. We sought to determine effect of antenatal corticosteroids on fetal bone metabolism and if effect is dose-dependent.

METHODS: Secondary analysis of randomized, placebo-controlled, multicenter trial. Women at < 32 weeks gestation at risk for spontaneous preterm delivery who remained pregnant after an initial course of corticosteroids were randomized to weekly repeat courses of betamethasone or placebo. Umbilical cord blood samples were collected at delivery. Plasma levels of carboxy terminal pro-peptide of type I pro-collagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) were determined by ELISA to assess rate of bone formation and resorption, respectively. Median values were calculated and comparisons made between groups receiving repeat study courses of betamethasone vs placebo, after first course of steroids, by using Wilcoxon Rank Sum Test. Analysis stratified according to number of repeat antenatal study courses of betamethasone or placebo (1 to 3 vs ≥ 4 courses).

RESULTS: 114 women were included in placebo group and 137 in betamethasone group. 41 women in placebo group and 46 in betamethasone group received 1-3 repeat study courses, while 73 and 91 women, respectively received ≥ 4 repeat study courses. Median umbilical cord blood ICTP levels were significantly lower (p = 0.014) in the betamethasone group compared with placebo group. There was no difference between the groups in median PICP levels. In the fetuses exposed to ≥ 4 repeat study courses, there was a significant difference between treatment groups in median ICTP levels (p=0.042) but there was no difference between treatment groups in the fetuses exposed to 1 to 3 repeat study courses (p=0.294).

CONCLUSION: Bone resorption but not formation is suppressed in fetuses exposed to multiple courses of antenatal betamethasone. The physiologic impact of this bone resorption is unknown. Up to 4 courses of antenatal betamethasone do not appear to affect fetal bone metabolism.

	PICP (µg/L) Placebo	PICP (µg/L) Betamethasone	P value	ICTP (µg/L) Placebo	ICTP (µg/L) Betamethasone	P value
All samples	479.1	480.2	0.567	57.9	55.0	0.014
1-3 courses	478.0	422.0	0.365	56.7	57.4	0.294
≥ 4 courses	480.1	531.0	0.169	58.6	53.4	0.042

679

Maturational Changes in Ovine Fetal Colonic Cholinergic Circuitry Parallels Plasma Glucocorticoid Surge. Jayaraman Lakshmanan,[1] Katrin S Lips,[2] Guong L Liu,[1] Michael G Ross.*[1] *[1]Dept. Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA; [2]Inst. for Anat. & Cell Biol, Justus-Liebig-University, Giessen, Germany.*

Objective**:** As the cholinergic system represents one of major effectors of gut motility, maturation of the fetal intestinal cholinergic system may contribute to *in utero* and newborn meconium passage. Glucocorticoids may accelerate cholinergic maturation, as antenatal glucocorticoid administration to the preterm ovine fetus augments cholinergic-stimulated colonic smooth muscle contractility. In fetal rabbits, intra-gastric administration of betamethasone, a synthetic glucocorticoid, induces preterm meconium passage. To examine the role of glucocorticoids in the maturation of fetal colon cholinergic circuitry, we quantified the expression of three cholinergic markers in the preterm and term ovine fetal colon, during periods when circulating fetal glucocorticoid levels are markedly different.

Method: Three cholinergic markers used were: 1. Peripheral choline acetyltransferase (pChAT), 2. Vesicular acetylcholine transporter (VAChAT), and 3. High affinity choline transporter (CHT-1). Immunostaining to pCHAT and CHT-1 were performed using rabbit polyclonal antiserum generated by one of our group (SL) and antibody to VAChAT was obtained from Sigma. Bouin's solution fixed, paraffin sections of preterm (118-120 days) and term (146-147days) ovine fetal distal colonic rings were subjected to immunohistochemsitry by avidin-biotin-perioxidase system and 3, 3'-diaminobenzidine (Sigma) as a chromogen. Percent of myenteric ganglia expressing cholinergic markers were determined by examining sections under microscope.

Results: The percent of myenteric ganglia expressing cholinergic markers in term fetuses (pCHAT: 40%, VAChAT: 27%, and CHT-1: 64%) was significantly ($p<0.05$) higher than those observed in preterm fetuses (pCHAT: 22%, VAChAT: 14% and CHT-1: 48%).

Conclusion: The marked increases observed in cholinergic markers in myenteric ganglia in fetal distal colon at term is possibly mediated by effects of endogenous glucocorticoid, levels of which peak at term. We speculate that glucocorticoids are the physiological hormones that regulate enteric cholinergic neural circuitry system, similar to their actions in central nervous system regions.

680

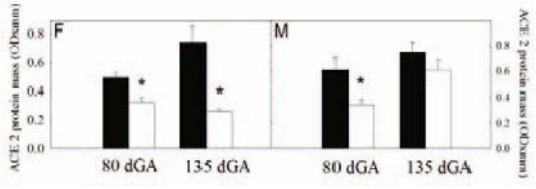
Antenatal Betamethasone Administration Decreases ACE 2 Protein in Sheep Kidney Cortex. Gretchen Koontz,[1] Mark Chappell,[2] Angela G Massmann,[1] Jie Zhang,[1] Jorge P Figueroa.*[1] *[1]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]Surgical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

Objective: Exposure to high levels of glucocorticoids (GC) in the perinatal period has been shown to induce hypertension later in life. The Renin-Angtiotensin-System (RAS) has a central role in fetal kidney development and in the regulation of blood pressure. The aim of this study was to further evaluate the acute and long term effects of a single-course of GC at 80 days gestation (dGA) on the expression of ACE1 and ACE2 in the kidney cortex (KC) and to assess whether gender-related differences are further affected by GC exposure.

Methods: Date-mated sheep were randomly assigned to receive a single course of GC [2 maternal IM doses of Beta (0.17 mg/Kg with a maximum of 12 mg) or vehicle 24-h apart] at 80 dGA. One group was delivered by C-section at 24h, another at 135 dGA and a third one allowed to deliver at term and euthanized at 0.8-1.5 yr of age. KC were obtained from fetuses and adults and

immediately frozen in liquid nitrogen. ACE1 and ACE2 were measured in KC using Western Blot. Results were expressed as Mean ± SEM and were analyzed by the t test. **Results:** ACE2 protein levels were significantly decreased in the KC of GC exposed female (F) and male (M) fetuses as compared to controls at 80 dGA ($p<0.01$). ACE2 protein levels remained significantly decreased in the KC of GC exposed females at 135 dGA ($p<0.01$), but were not significant in male fetuses ($p=0.28$). The effect of GC on ACE2 levels was not present in the adult animals. No significant differences were seen in ACE1 at any of the gestational ages evaluated.

Conclusion: The changes in ACE2 protein expression parallel our previous findings in mRNA. An imbalance of Ang II and Ang (1-7) levels early in development may impact the RAS and play a role in altering the early development of the fetal KC. These alterations in kidney development as well as the changes in intrarenal RAS appear to be influenced by gender and may be responsible for later development of hypertension. HL 68728; HD P01 HD04784.



681

Knock Down of ACTH Receptor (ACTH-R) Inhibits StAR Expression Cortisol Production in Ovine Fetal Adrenocortical Cells. Yixin Su,[1] James C Rose.[*2] *[1]Department of Obstetrics and Gynecology, Wake Forest University Health Science, Winston-Salem, NC, USA; [2]Department of Physiology/ Pharmacology, Wake Forest University Health Science, Winston-Salem, NC, USA.*

Objectives: The increase in fetal plasma cortisol that precedes birth in sheep is accompanied by increases in adrenocorticotropin receptor (ACTH-R) expression in the fetal adrenal and in adrenal responsiveness to stimulation. To gain more insight into the importance of changes in ACTH-R expression on adrenal function, we used small interfering RNA (siRNA) targeted to the ovine ACTH-R to reduce receptor expression and studied responses to stimulation in ovine adrenal cells. We studied fetal cells from late gestation, after responsiveness has increased, and adult cells to determine if maturation would influence the impact of receptor expression suppression.

Methods: We used recombinant plasmids producing hairpin small interfering RNA (ShRNA) to target ovine ACTH-R to knock-down ACTH-R expression in ovine adrenocortical cells. Fetal and adult cells were obtained, dispersed, transfected with receptor targeted siRNA or scrambled siRNA and subsequently stimulated with ACTH. The cyclic AMP (cAMP) and cortisol in the supernatant were measured by enzyme linked immunoassay (EIASA) or radioimmunoassay (RIA). RNA samples were subjected to RNase Protection Assay (RPA) to measure the ACTH-R or StAR mRNA levels. Western blots were used to measure protein level of ACTH-R and StAR. Data were analyzed by analysis of variance.

Results: The ability of ACTH to upregulate its receptor or steroid acute regulatory protein (StAR) was attenuated in fetal ($p<0.01$) and adult cells ($p<0.01$) by siRNA treatment; the blockade was more pronounced in the adult cells ($p<0.01$). The siRNA treatment also blocked the cAMP response to ACTH in fetal ($p<0.001$) and adult ($p<0.05$) cells. This was accompanied by marked reductions in cortisol responses in both ($p<0.001$ and $p<0.01$, respectively).

Conclusion: These data suggest that upregulation of the ACTH receptor expression in late gestation is essential for the increase in adrenal steroidogenic capacity occurring then. The data also indicate that a reduction in ACTH receptor expression blocks the ability of the peptide to stimulate early steps in the steroidogenic pathway in both fetal and mature adrenal cells.

Supported by NIH grant HD 11210.

682

Impact of *In Vitro* Embryo Culture on Fetal Adrenal Growth and Steroidogenic Gene Expression in the Sheep in Late Gestation. Olivia Wyss,[1,2] Severence M MacLaughlin,[1,2] Simon K Walker,[3] Caroline I McMillen.[1,2] (SPON: David M Olson). *[1]Discipline of Physiology, The University of Adelaide; [2]Samson Institute, The University of South Australia; [3]SARDI, Adelaide, South Australia, Australia.*

Introduction: In vitro embryo culture (IVC) results in fetal growth restriction and preterm delivery the human and rodent whilst in sheep IVC in the presence of serum is associated with large offspring syndrome (LOS) and a delayed parturition. LOS has been shown to be associated with a decrease in expression of IGF-2R, the IGF-2 clearance receptor, in fetal tissues. An increase in fetal adrenal growth and steroidogenesis in late gestation is essential for the normal timing of parturition in the sheep. It is not known, however whether IVC alters fetal adrenal growth and/or steroidogenic capacity.

Objective: To test the hypotheses that IVC results in: (1) a decrease in adrenal IGF-2R expression and (2) a decrease in cytochrome P450 17α-hydroxylase (CYP17), in the fetal adrenal.

Methods: Embryos were collected 24h after artificial insemination of superovulated donor ewes, which were subsequently transferred to one of 3 treatment groups: -to intermediate ewes until day 7 [embryo transfer (ET) group (singletons (S)=5, twins (T)=6)]; -to an in vitro culture of synthetic oviductal fluid either without [IVCNS (S=7, T=14)] or with human serum [IVCHS (S=7, T=4)] until day 6. Embryos were then transferred to final recipient ewes. Naturally mated (NM) ewes were used as controls [S=5, T=8]. At 144/145d, ewes were killed, and fetal adrenals were collected and weighed. IGF-2, IGF-2R and CYP17 mRNA expression was determined using rt PCR.

Results: The relative adrenal weight was greater ($P<0.05$) in the IVCNS (0.102±0.03g/kg) group when compared to ET (0.97±0.03g/kg) and IVCHS groups (0.092±0.03 g/kg) but not compared to the NM group. CYP17 mRNA expression was increased ($P<0.05$) in the IVCNS (4.7±1.3) compared to the NM (3.1±0.9) groups. IGF-2R mRNA expression was lower ($P<0.02$) in the IVCHS group (0.022±0.007) compared to the ET (0.047±0.014), IVCNS (0.043±0.009) and NM (0.046±0.014) groups.

Conclusion: A novel finding of this study is that the in vitro embryo culture systems similar to those used for ART, ie IVCNS, resulted in an increase in adrenal growth and CYP17 expression. In contrast, IVCHS results in a decrease in adrenal IGF2R expression. Thus, mechanisms regulating the prepartum activation of the fetal adrenal in late gestation may be altered by events early in embryo development.

683

Antenatal Glucocorticoid Therapy Induces Vascular Remodelling and Oxidative Stress in the Peripheral Vasculature in Fetal Sheep. S Waise, AM Shelley, FBP Wooding, AJ Forhead, AL Fowden, Dino A Giussani.[*] *Physiology, Development & Neuroscience, Cambridge, United Kingdom.*

Antenatal glucocorticoid therapy (AGT) reduces infant morbidity in pregnant women with threatened preterm delivery. However, there is growing concern whether AGT elicits unwanted side-effects. Glucocorticoids depress NO activity, promote endothelial dysfunction, and increase peripheral resistance and arterial pressure in the sheep fetus (Molnar et al. *AJP* **283**:R561,2002; Fletcher et al. *J Physiol* **545**:649, 2003). The molecular basis of these effects remains unknown. We hypothesise that the actions of AGT on the fetal cardiovascular system are due to reduced NO bioavailability secondary to enhanced ROS generation. We investigated the effects of a single course of AGT on vessel structure and vascular oxidative stress in fetal sheep.

Methods: Pregnant ewes were treated with dexamethasone (2 x 12 mg in 2 ml of saline i.m. 24 h apart; n=6) or saline (2 x 2 ml saline i.m. 24 h apart, n=5) at 124±1 days of gestation. Two days later, animals were humanely killed and the fetal carotid artery, descending aorta and femoral artery were isolated and fixed. Vessels were processed for calculation of wall:lumen area ratio, elastin strand density, expression of smooth muscle actin and nitrotyrosine.

Results: Relative to controls, maternal dexamethasone treatment showed increased vascular wall thickness and reduced elastin density in the fetal femoral artery and descending aorta but not in the carotid artery (Fig. 1). Dexamethasone also increased the total expression of smooth muscle actin and nitrotyrosine in fetal femoral vessels only (Fig. 2).

Conclusion: Antenatal glucocorticoid therapy induces regional changes in structure and oxidative stress in the fetal vasculature.

Supported by The Physiological Society, UK.

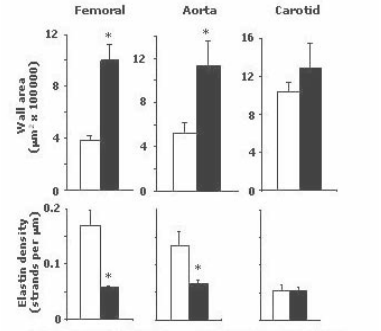


Fig.1. Mean+SE for fetal vessels after maternal dexamethasone (n=6,■) or saline (n=5, □) treatment. *P<0.05, t test.

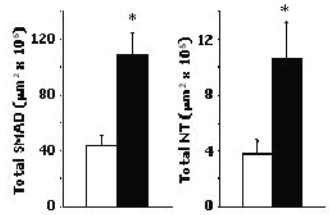


Fig.2. Mean+ SE for femoral arteries from dexamethasone (n=6,■) or saline (n=5, □) pregnancies. Total smooth muscle actin (SMAD) and total nitrotyrosine (NT) were calculated by multiplying uptake of antibody by wall area. *P<0.05, t test.

684

Corticotrophin Releasing Factor Is a Fetal Gut Hormone. Jayaraman Lakshmanan, John D Richard, Guong L Liu, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Studies in adult rats indicate that corticotrophin releasing factor (CRF) is an important modulator of gastrointestinal (GI) motility and secretion. CRF of both central and peripheral origin has been implicated to modulate adult gastrointestinal stress responses. In support of peripheral CRF pathway, mucosal endocrine cells have been identified to synthesize CRF in human colon. Utilizing a hypoxic stress paradigm we recently induced in utero meconium passage in term fetal rats. Based on the marked increases in plasma CRF levels in meconium stained fetuses, we hypothesized that stress-induced in utero meconium passage is mediated by the peripheral CRF pathway. In support of our hypothesis we demonstrated CRF-receptors expression in fetal rat gastrointestinal smooth muscle and epithelial layers. In the present study we sought to identify the peripheral source(s) of CRF by examining rat fetal GI tract for CRF localization.

Method: Whole GI tracts were dissected from rat fetuses et 21 (n=11), fixed in Bouin's solution and paraffin embedded. Five micron sections were cut and immunostaining performed by Vector's ABC regimen using standard protocol. Sections were incubated either with rabbit polyclonal antibody to CRF (1:300, Peninsula Laboratories) or non-immune rabbit IgG at similar dilution. Immunoreactive materials on the sections were identified as brown staining using 3,3' diaminobenzidine as chromogen. Slides were counterstained with haematoxylin solution, coverslipped and examined under microscope.

Results: CRF-antibody elicited strong positive immunostaining in isolated endocrine cells dispersed through out epithelium. CRF expressing enteroendocrine cells predominantly reside on the villi and are less abundant in crept regions. The number of immunopositive endocrine cells greatly varied between lumens. In addition to endocrine cells, luminal epithelial cell surface also exhibited positive staining but the staining intensity is far less than that of endocrine cells. Faint positive immunostaining was noticed in muscle layers.

Conclusion: CRF can be localized in enteric endocrine cells in rat fetus at e21. We speculate that CRF synthesized by the fetal enteric endocrine cells may function as an important modulator of gastrointestinal motility, secretion and blood flow as in adult rats.

685

CRH-R1 Antagonist Attenuates Ovine Adrenal Cortical Cell Responses to ACTH. Nancy K Valego, James C Rose.* *Center of Research for Ob/Gyn, Wake Forest University Medical School, Winston-Salem, NC, USA.*

OBJECTIVE: As gestation progresses in fetal sheep, the adrenal gland is increasingly sensitized to ACTH resulting in the cortisol surge leading to parturition. Since CRH is abundant in human placenta and CRH receptor types 1 & 2 have been demonstrated in human adrenal cortex, CRH has been implicated in parturition. CRH treatment of cultured human definitive and transitional zone cells results in concentration-related cortisol secretion, and CRH pre-treatment enhances response to ACTH. Last year we reported that, in dispersed 101 days gestation (dg) fetal ovine adrenal cortical (AC) cells, CRH, alone, had minimal effect on secretion but attenuated the cortisol response to ACTH. Our objective in this study was to further investigate the interaction of CRH and ACTH using the specific CRH-R1 antagonist, antalarmin (ANT), on fetal and adult ovine AC cells.

METHOD: Adrenals were removed from adult and 101 dg fetal sheep at necropsy and AC cells dispersed. After 48 hrs., cells were pretreated for 30 mins. with vehicle (2% DMSO) or ANT (10 μM in 2% DMSO) and then stimulated with ACTH or forskolin (FSK). Medium was removed 2.5 hrs. later for cyclicAMP measurement by EIA or after 24 hrs. for cortisol measurement by RIA. After 24 hour treatment, cell viability was determined by CellTiter 96 Aqueous One Solution Cell Proliferation Assay. Comparisons were done with t-test. Except where noted, n=4 or 5.

RESULTS:

Age	Secretagogue	Cortisol: ng/ml/24hr.		CyclicAMP: pmol/2.5 hr.	
dg		No antagonist	ANTALARMIN	No antagnist	ANTALARMIN
101	ACTH @ 150 nM	169.4 ±35.3 (n=2)	2.64 ±0.94 (n=9)	15.2 ±5.7 (n=2)	5.6 ±1.0 (n=5)
Adult	ACTH@ 50nM	579.2 ±77.3	432.9 ±87.1*	414.9 ±101.0	271.6 ±87.8*
Adult	FSK@ 10^{-6}M	318.3 ±34.5	208.2 ±31.0**		

* p<.05; ** p=.001

ACTH-induced 24 hour cortisol secretion is attenuated by ANT in fetal and adult AC cells.

FSK-induced 24 hour cortisol secretion is attenuated by ANT in adult cells.

ACTH-induced 2.5 hour cyclicAMP was attenuated by ANT in fetal and adult cells.

ACTH-induced 24 hour cell proliferation was unchanged by ANT

CONCLUSION:

The CRH-R1 antagonist, antalarmin, attenuates ACTH-induced cortisol and cyclicAMP secretion from dispersed fetal and adult ovine AC cells as well as FSK-induced cortisol secretion from adult AC cells. Attenuation is not the result of cell death. It appears that this antagonist has an effect distal to the ACTH receptor.

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686

Differential Steroid Pathway Responses to Adrenocorticotropin Hormone and Corticotropin-Releasing Hormone in Human Fetal Adrenal (HFA) Cell Cultures. Daniel R Christie,[1] Barbara Staton,[1] William E Rainey,*[2] C Richard Parker, Jr.[1] [1]*Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL, USA;* [2]*Department of Physiology, Medical College of Georgia, Augusta, GA, USA.*

Objective: Our objective was to compare the 24-hour time courses for secretion of cortisol (F) and dehydroepiandrosterone (DHEA) in midtrimester whole HFA glands when stimulated by either adrenocorticotropin hormone (ACTH) or corticotrophin releasing hormone (CRH).

Methods: Cells harvested from several midtrimester whole HFA glands were incubated in 96 well plates (approx. 280 x 10^3 cells/well). After three days incubation, each culture was supplemented with control medium, medium plus 10nM ACTH, or medium plus 10nM CRH, n = 6 wells per condition. The time points analyzed were 1, 3, 6, 12, and 24 hrs. Concentrations of F and DHEA were determined by enzyme immunoassays.

Results: DHEA production was significantly higher than controls in both ACTH and CRH stimulated cells beginning at the initial time point (Figure 1). F secretion by HFA cells stimulated with ACTH was significantly higher than control by the six hour time point. F secretion in cells stimulated with CRH was similar to control until the 24 hour time point (Figure 2) The ratio of DHEA to F produced rose up to 12 hrs and was significantly higher in the ACTH and CRH stimulated cells versus control until the 24 hour time point at which time the ratios were reduced to control levels (not shown).

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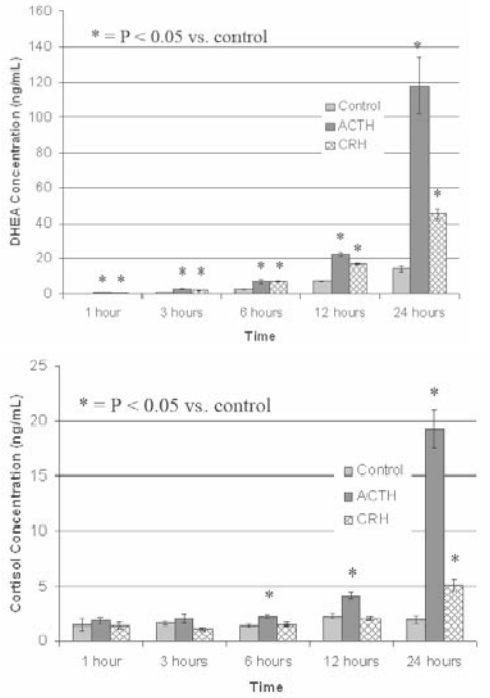
Conclusions: We conclude that in the midtrimester HFA, secretion of DHEA occurs sooner in response to stimulation than does cortisol, suggesting that intermediate enzymes in the DHEA pathway are already present, whereas those necessary for F production are not being expressed until after stimulation.



687

The Outer Zone of the Human Fetal Adrenal Gland Is a Site of Angiogenesis. Hitoshi Ishimoto,[1] Satoshi Asai,[1] Takayuki Higuchi,[1] Kazuhiro Minegishi,[1] Mamoru Tanaka,[*1] Yasunori Yoshimura,[*1] Robert B Jaffe.[*2] *[1]Dept. of Ob/Gyn, Keio Univ, Tokyo, Japan; [2]Center for Reproductive Sciences, Univ. of California, San Francisco, San Francisco, CA, USA.*

Objective: While the inner fetal zone (FZ) of the midgestation human fetal adrenal (HFA) produces dehydroepiandrosterone sulfate, a precursor of placental estrogen, the function of the outer definitive zone (DZ) is less clear. We have proposed that the DZ phenotype is that of a pool of progenitor cells, many of which are mitotically active. The adrenal gland is one of the most vascularized organs in the fetus and undergoes rapid growth during midgestation. Recently we studied expression and ACTH regulation of an important family of vascular endothelial cell-specific angiogenic factors, angiopoietin-1 and -2 (Ang1 and Ang2) in the HFA. Ang2 was predominantly localized in the periphery of the gland. Ang2 expression has been shown to be restricted to sites of vascular remodeling. In this study, therefore, we tested the hypothesis that the periphery of the HFA is a site of angiogenesis.

Methods: Midgestation HFA's (16-23wk) were used. Proliferating endothelial cells were detected by double immunofluorescence with the endothelial cell markers, CD31 and Ulex lectin, and a proliferation marker, Ki67. Zonal differential expression of Ang1, Ang2, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) was investigated by laser-capture microdissection and real-time quantitative RT-PCR (qPCR). Regulation of Ang1, Ang2, and VEGF mRNA was studied by qPCR in isolated HFA cells.

Results: 1) Double immunostaining demonstrated that proliferating endothelial cells were limited at the DZ and DZ/FZ border. 2) Ang2 mRNA was primarily localized in the DZ whereas Ang1 mRNA was primarily in the FZ (Ang2/Ang1 mRNA ratios: 9.3 ± 2.3, DZ; 1.2 ± 0.2, FZ; mean ± SE, $p < 0.05$, $n = 5$). VEGF and bFGF mRNA levels were also higher in the DZ. 3) Among locally-produced growth factors implicated in HFA development, bFGF (10 ng/ml) induced Ang2 mRNA by 4-fold in both zones (FZ: $p < 0.01$; DZ: $p < 0.05$, at 24h), but not Ang1 nor VEGF mRNA.

Conclusions: These results suggest that angiogenesis occurs at the periphery of the HFA. As Ang2 renders endothelial cells responsive to angiogenic stimuli such as VEGF and bFGF, predominant expression of these factors by the DZ further supports this notion. The zonal differential expression of Ang2 may be explained, in part, by the parallel pattern of bFGF expression.

688

Syndecan-3 Localization Is Limited to the Definitive Zone of the Human Fetal Adrenal Gland: Implications for Adrenal Development. Hitoshi Ishimoto,[1] Satoshi Asai,[1] Takayuki Higuchi,[1] Kazuhiro Minegishi,[1] Mamoru Tanaka,[*1] Yasunori Yoshimura,[*1] Robert B Jaffe.[*2] *[1]Dept. of Ob/Gyn, Keio Univ, Tokyo, Japan; [2]Center for Reproductive Sciences, Univ. of California, San Francisco, San Francisco, CA, USA.*

Objective: Rapid growth of the human fetal adrenal gland (HFA) at midgestation is supported by an active proliferative drive observed in the periphery of the gland, i.e., the definitive zone (DZ). Before the third trimester, DZ cells are not capable of producing steroids while they exhibit numerous mitotic figures. Our recent finding of DZ cell-selective proliferative effects of midkine (MK), a heparin-binding growth factor, provides insight into the mechanisms by which DZ cells maintain their proliferative phenotype in vivo. MK binds to and signals through several proteoglycans. We hypothesized that an MK receptor that has DZ-specific expression may be involved in the DZ-selective mitogenic effects of MK. In this study, we investigated the expression and localization of putative MK receptors in the HFA.

Methods: HFA's (16-23wk) were used. Expression of transcripts encoding putative MK receptors was examined by RT-PCR. Immunocytochemical studies were performed to localize the receptors. Zonal differential mRNA expression was evaluated by laser-capture microdissection (LCM) and real-time quantitative RT-PCR (qPCR). ACTH regulation of the receptors of interest was further studied by qPCR in isolated HFA cells.

Results: Among the receptors implicated in MK signaling, protein-tyrosine phosphatase ζ and anaplastic lymphoma kinase were not expressed in midgestation HFA's. Low-density lipoprotein receptor-related protein (LRP) was expressed; however, significant immunoreactive LRP was only seen in the capsule, not in cortical cells. Syndecan-3 and -4 (SDC3 and SDC4), heparan sulfate proteoglycans, were expressed in the HFA. SDC4 was localized abundantly on cortical cell membranes throughout the gland. In contrast, immunoreactive SDC3 was limited to the DZ. The zonal differential expression of SDC3 mRNA was further confirmed by LCM and qRTPCR. However, addition of ACTH did not change SDC3 mRNA in isolated DZ cells.

Conclusions: With its DZ-specific localization, SDC3 may be a receptor that mediates DZ-selective mitogenic effects of MK. SDC3 also is an important coreceptor for basic fibroblast growth factor, a known potent growth factor for adrenocortical cells. Taken together, SDC3 may play a role in maintaining the proliferative phenotype of the DZ.

689

ROS Alter the Sensitivity of the Adenocortical Response to Acute Hypoxic Stress Via Changes in NO Bioavailability in Fetal Sheep. Avnesh S Thakor, Dino A Giussani.[*] *Physiology, Cambridge, United Kingdom.*

Reactive oxygen species (ROS) modulate the pituitary-adrenal axis via intracellular signalling mechanisms (Xu et al. *Neuroscience* **126**:313, 2004). ROS can also alter nitric oxide (NO) bioavailability, which, in turn, regulates the pituitary-adrenal axis either as a neurotransmitter and/or neuromodulator (Riedel. *Z. Rheumatol.* **59**:36, 2000). It remains completely unknown in both pre- and postnatal life whether ROS affect the pituitary-adrenal response to stress. We investigated the effects on the fetal ACTH and cortisol responses to acute hypoxic stress of two well-known antioxidants, vitamin C and melatonin.

Methods: Under anesthesia, 12 fetal sheep (0.8 gestation) were instrumented with vascular catheters. Five days later, fetuses were randomly allocated into 2 groups (n=6) and subjected to 1.5 h normoxia, 0.5 h hypoxia and 1 h recovery during saline infusion, antioxidant treatment (vitamin C: 25 $mg.min^{-1}$; melatonin: 0.15 $\mu g.min^{-1}$) and antioxidant treatment during NOS blockade. Fetal arterial blood was taken for measurement of blood gases and plasma concentrations of ACTH and cortisol (RIA).

Results: Basal plasma ACTH and cortisol concentrations were similar in all fetuses and were not affected by saline infusion or antioxidant treatment either alone or during NOS blockade. Hypoxia produced similar falls in P_aO_2 in all fetuses (~21 to 10 mmHg). During saline infusion, hypoxia induced significant increments in plasma ACTH and cortisol concentrations. In contrast, hypoxia-induced increments in plasma ACTH and cortisol were diminished and enhanced, respectively, during fetal antioxidant treatment ($P<0.05$). Correlation analysis of individual ACTH and cortisol values for all fetuses showed an enhanced adrenocortical sensitivity to ACTH, during both vitamin

C and melatonin treatment relative to saline infusion (P<0.05). Antioxidant treatment during NOS blockade recovered the magnitudes of the ACTH and cortisol responses (Fig.1).

Conclusion: In the sheep fetus, antioxidants increase the sensitivity of the adenocortical response to hypoxic stress by increasing NO bioavailability.

Journal of Experimental Pathology and The Lister Institute.

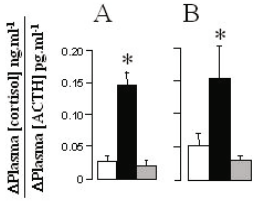


Fig. 1. Bars represent the mean±S.E.M. for the gradients from all individual fetal paired ACTH and cortisol relationships during basal and hypoxemic conditions, with saline infusion (□; n=6), antioxidant treatment (■; n=6) or treatment with antioxidant during NOS blockade (grey bars; n=6). A, melatonin experiment; B, vitamin C experiment. *P<0.05 vs all.

690

Effect of Cortisol Infusion in the Late Gestation Hypothalamo-Pituitary Disconnected Sheep Fetus on Pituitary Responsiveness to Arginine Vasopressin. Luke C Carey,[1] Stephen B Tatter,[2] James C Rose.*[1] *[1]Center of Research for Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]Neurosurgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

The late gestation fetal plasma cortisol surge stimulates development of the lung and other organ systems. The precise mechanisms underlying this surge are unclear. At the pituitary level, corticotrophs become increasingly responsive to arginine vasopressin (AVP) with advancing gestation. We have recently found that this may due in part to corresponding increases in signal transduction following stimulation. Furthermore these ontogenic changes in signal transduction are prevented by hypothalamic-pituitary disconnection (HPD), which also impedes the cortisol surge. This led us to hypothesize that cortisol may be involved in mediating changes in pituitary responsiveness to AVP. To further investigate this, we infused late gestation HPD fetuses with cortisol (HPD + R) and examined pituitary responsiveness in vitro. HPD was performed at around 120 days of gestation (dGA), and a 3 day cortisol infusion (1.2 μg/kg/min) was initiated on 135-137 dGA (Control HPD fetuses were not cortisol infused). Pituitaries were then immediately dissected, dispersed and plated at 2.0×10^5 cells per well in 48 wells plates. After 48h, cells were stimulated with 100nM AVP for 2h, and the medium collected for ACTH analysis. Plasma cortisol concentrations increased rapidly within the first 6h following infusion (5.2 ± 1.7 to 29.7 ± 4.9 ng/ml), but did increase further. In non-infused fetuses, cortisol concentrations were 4.8 ± 0.6 ng/ml at 72h. Cells from HPD + R fetuses secreted significantly more ACTH than those from HPD fetuses (% increases from baseline were 45.5 ± 9.3 and 17.7 ± 3.7 respectively). For reference, increases in cells from sham/control fetuses are around 60%. These findings support the idea that cortisol plays a role in mediating pituitary responsiveness to AVP stimulation in the late gestation fetus.

Supported by NIH Grant HD 11210.

691

Evidence for Racial Differences in Corticosteroid Metabolism in Normal Pregnancy. Angela G Duke, Christine W Mansfield, Barbara A Staton, C Richard Parker.* *Dept OB GYN, University of Alabama at Birmingham, Birmingham, AL, USA.*

Hypothesis: Racial disparities exist such that African American women and their newborns are at highest risk for adverse pregnancy outcomes, particularly preterm delivery and low birthweight. Data from several experimental models are suggestive that excessive cortisol synthesis or transplacental passage to the fetus may play a role in prematurity, growth retardation, and even adverse health conditions in postnatal life. We sought to determine if evidence exists for increased intrauterine exposure of African American infants to cortisol in otherwise uncomplicated pregnancies.

Methods: Maternal serum was collected at admission for elective term cesarean section deliveries from 23 African American (AA) women and 14 non-Hispanic Caucasian (white) women; umbilical venous serum also was collected at delivery from their singleton infants. We measured total cortisol levels in these maternal and umbilical cord samples by RIA.

Results: Gestational age at delivery and birthweights of the 2 groups were similar. Cortisol levels in the AA women (27.6 +/- 1.3 ug/dl) were slightly lower than those in the white women (29.7 +/- 2.3 ug/dl). Umbilical venous serum cortisol levels in the AA infants (6.9 +/- 0.5 ug/dl) were 23% higher than in the white infants (5.6 +/- 0.9 ug/dl). In addition, the maternal/umbilical venous cortisol ratio in the white pregnancies (6.8 +/- 0.9) was 50% higher (P= 0.016) than that of the AA pregnancies (4.5 +/- 0.4). Such a disparity is consistent with a significantly increased transplacental passage of maternal cortisol to the fetal vasculature or increased synthesis in the fetal adrenal in the AA pregnancies.

Conclusions: We found evidence suggestive of increased exposure of the feto-placental unit to cortisol in pregnancies of AA women. The altered maternal to fetal ratio of cortisol in AA pregnancies could result from decreased levels or activity of placental 11 hydroxysteroid dehydrogenase type 2, which serves to inactivate cortisol by conversion to cortisone, or increased 11 hydroxysteroid dehydrogenase type 1, which mainly converts cortisone to cortisol. An alternate explanation would be increased fetal adrenal cortisol synthesis in the AA pregnancies. Increased levels of cortisol in the feto-placental unit in AA pregnancies could contribute to the racial disparities in pregnancy outcomes.

692

Global DNA Methylation (ME) in the Fetal (F) Sheep Liver Increases in the Second Half of Gestation. Alexander Unterberger,[5] B Hess,[1,2] LA Cox,[4] M Szyf,[5] Peter W Nathanielsz,*[1,3] SP Ford.[1,2] *[1]Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY, USA; [2]Animal Science, University of Wyoming, Laramie, WY, USA; [3]Center for Pregnancy and Newborn Research, UTHSCSA, San Antonio, TX, USA; [4]Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA; [5]Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada.*

Approx 70-80% cytosine rings in dinucleotide sequence CG are methylated (ME). ME CGs program genome expression by silencing genes in gene and tissue specific patterns. ME patterns are generated during development by ME and deME reactions. Disruption of gene ME is involved in cancer and other diseases. **Hypothesis:** During gestation pluripotent cells differentiate into terminal phenotypes characterized by a restricted gene expression repertoire. We hypothesized that global gene ME increases with gestation. We also hypothesized that 50% global MNR would decrease global ME. We determined 1) changes in global ME in fetal (F) sheep liver (L) in the 2nd half of gestation and 2) if 50% global maternal nutrient restriction (MNR) alters global ME. **Methods:** Control fed (CF, n = 12, 100% NRC requirements) or NR (n= 11, 50% NRC requirements) diets began at 28 days gestation (dG). 8 CF and 6 NR ewes were necropsied at 78 dG and 4 CF and 5 NR ewes at 135 dG. F L (left lobe) was flash frozen in liquid N,stored at -80°C, DNA extracted and 5-methylcytosine in CG dinucleotides quantified by nearest-neighbor analysis [3]. Data (M + SEM) analyzed by two way ANOVA. **Results:** F body and L wts for 78dG CF and NR were 272 ± 11 and 16.6 ± 1.0g vs. 211 ± 11 and 13.4 ± 0.9g, respectively. F body and L wts for 135dG CF and NR groups were 5358 ± 286 and 143.2 ± 10.2 vs. 5197 ± 174 and 157 ± 5.0g, respectively. Global ME was 53.5 + 2.9 and 59.6 + 3.4% in CF and NR respectively (ns) at 78 dG and 83.5 + 2.53% and 74.8 + 3.26% at 135 dG in CF and NR respectively ($p = 0.08$). Global ME increased between 78 and 135 dG in CF and NR ($p < 0.05$). **Conclusions:** This is the first evaluation of global ME in F sheep a common model of developmental programming [2]. The reduction of global ME by 8.7% as a result of 50% MNR throughout pregnancy is consistent with postnatal MNR rats showing a 20% decrease in GR and PPAR gamma gene ME [1]. References: 1. Lillycrop et al J Nutr. 2005;135:1382-86.2. Armitage et al J Physiol 2004;561:355-77.3. Ramshahoye 2002. Methods Mol Biol. 200: 9-15.

693

Ontogeny of Genes Potentially Important for Lung Liquid Movement during Fetal Life. Nathan M Jesse,[1] Marcela von Reitzenstein,[2] Maureen Keller-Wood.*[2] *[1]Department of Pediatrics, University of Florida, Gainesville, FL, USA; [2]Department of Pharmacodynamics, University of Florida, Gainesville, FL, USA.*

Objective: Lung liquid secretion throughout most of gestation is critical for normal lung development; in anticipation of the transition to extrauterine life, the lung switches to a reabsorptive phenotype late in gestation. We have previously reported that expression of the epithelial sodium channel α-subunit ($ENaC_\alpha$) is increased in the lungs of 145d fetuses and neonates; in this study we include term fetuses of ewes in labor. We also evaluated the ontogeny of other target genes purported to be important for shifts in fetal lung fluid.

SATURDAY

Methods: RNA was isolated from lung tissue from 80, 120, 130, and 145day gestation ovine fetuses, and fetuses of ewes in labor (term) as well as one- and seven day old lambs, and real-time PCR was performed. Gene expression was normalized to β-actin expression.
Results: The chloride channel ClC2 mRNA showed ~2-fold greater expression at 80d than at 145d or in one- and seven-day lambs. The mRNA for the other major chloride channel in lung, CFTR, also showed significantly greater expression at 80d than at any other age. $ENaC_{\alpha}$ showed a significant increase in mRNA expression at 145d and at term compared to 80d (~15-16-fold); its expression decreased postnatally. The water channel aquaporin1 (AQP1) mRNA expression increased ~9-fold from 80d to a peak at term. AQP5 mRNA showed significantly greater expression at all gestational ages compared with 80d, peaking at 145d. There were increases of ~300-fold at 145d, ~170-fold at term, and ~120-150 fold postnatally as compared to 80d.
Conclusions: Gene expression followed expected patterns based on the role of the channels in lung liquid movement. Lung fluid secretion is driven by a chloride gradient. Our data, which show that ClC-2 and CFTR mRNA are more highly expressed early in gestation, suggest a possible role in the establishment of the chloride gradient. In contrast, reabsorption of fluid depends on the establishment of a sodium gradient. Water then follows sodium passively, paracellularly or through aquaporins. $ENaC_{\alpha}$ was expressed more abundantly late in fetal life, consistent with its accepted role in fluid reabsorption. AQP1 and AQP5 were also expressed more abundantly late in gestation than at 80d or postnatally, suggesting a possible role in the reabsorption of luminal water across the lung epithelium.

694

Organ and Age Specific Changes in IGF-1 Receptor Expression in Intrauterine Growth Restricted Newborns. Darran N Tosh,[1] Robert H Lane,[2] Isabella C McMillen,[3] Michael G Ross,[*4] Mina Desai.[4] *[1]Dept. of Physiology, Univ. of Adelaide, Adelaide, Australia; [2]Dept. of Pediatrics, Univ. of Utah, Salt Lake City, UT, USA; [3]Sansom Research Institute, Univ. of South Australia, Adelaide, Australia; [4]Dept. Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*
Objective: Maternal food restriction (MFR) during pregnancy causes IUGR offspring that when fed ad libitum from birth demonstrate catch-up growth by 3 weeks. Asymmetric growth is characteristic of IUGR and autocrine secretion of the growth factor IGF-1 may play a role in tissue specific growth. Despite reduced relative hepatic size at birth, liver size normalizes by 3 wks of age. We therefore sought to determine whether IGF-1 and IGF-1 receptor (IGF-1R) are differentially expressed in IUGR liver and whether these changes were age dependent.
Methods**:** Control Sprague Dawley dams (n=6) received ad libitum food, study dams (MFR, n=5) were 50% food-restricted from pregnancy day 10 to 21 resulting in IUGR newborns. At birth litter size was culled to 4 males and 4 females. All pups were nursed by dams fed ad libitum and were weaned to ad libitum feed. Plasma along with liver and kidney and were collected at gestational day 20 (E20), day 1 and 3 weeks. Plasma levels of IGF-1 were measured by RIA and IGF-1 and IGF-1R mRNA levels were quantified by Real-time PCR.
Results: Plasma IGF-1 was decreased in IUGR offspring at day 1(% of control: 89.8%, $p<0.01$) but not at 3 weeks. Conversely, in IUGR liver, IGF-1R mRNA was not different at E20 and day 1 but subsequently decreased at 3 weeks (66.4%, $p<0.05$). In contrast, IUGR kidney IGF-1R mRNA was significantly increased at E20 (188.1%, $p<0.05$), though no difference was evident at day 1 and 3 weeks of age. No change was seen in IUGR liver or kidney IGF-1 mRNA levels at E20, day 1 and 3 weeks.
Conclusion**:** IUGR alters IGF-1R mRNA levels in an organ and age specific manner. We speculate that IUGR alterations to the IGF system at critical periods of organ development may contribute to newborn catch-up growth and the later development of the metabolic syndrome.

695

Noninvasive Prenatal Detection of Fetal Trisomy 18 by Epigenetic Allelic Ratio Analysis in Maternal Plasma: Theoretical and Empirical Considerations. Tak Yeung Leung,[1] Yu Kwan Tong,[2] Chunming Ding,[3] Rossa Chiu,[2] Ageliki Gerovassili,[4] Stephen Chim,[1] Tse Ngong Leung,[1] Tze Kin Lau,[1] Kypros Nicolaides,[4] Dennis Lo.[2] (SPON: Carl Philip Weiner). *[1]Obstetrics and Gynecology, The Chinese University of Hong Kong, Hong Kong, Hong Kong; [2]The Chinese University of Hong Kong, Hong Kong, Hong Kong; [3]Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, Hong Kong; [4]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, United Kingdom.*
BACKGROUND: The discovery of cell-free fetal DNA in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis. However, the use of maternal plasma fetal DNA for the direct detection of fetal chromosomal aneuploidies has not been reported. We postulate that the aneuploidy status of a fetus could be revealed by an epigenetic allelic ratio approach, i.e., by analyzing the allelic ratio of a single-base variation present within DNA molecules exhibiting a placental-specific epigenetic signature in maternal plasma.
METHODS: Placental-derived fetal-specific unmethylated maspin promoter sequences on human chromosome 18 were detectable in placental-maternal DNA mixtures and in maternal plasma by bisulfite modification followed by methylation-specific PCR (MSP) and primer extension. The ratios between the extension products of the 2 alleles were calculated for heterozygous placentas, placental-maternal blood cell DNA mixtures, and maternal plasma samples. The allelic ratios were compared between pregnancies carrying trisomy 18 and euploid fetuses.
RESULTS: The epigenetic allelic ratios of all tested trisomy 18 samples deviated from the reference range obtained from euploid samples (placental DNA, 1.135 to 2.052; placental-maternal DNA mixtures, 1.170 to 1.985; maternal plasma, 0.330 to 3.044; without skew correction on the raw mass spectrometric data). A theoretical model was established and validated that predicted that a minimum of 200 copies of genomic DNA after bisulfite conversion were required for distinguishing euploid and aneuploid fetuses with confidence.
CONCLUSION: Epigenetic allelic ratio analysis of maternal plasma DNA represents a promising approach for noninvasive prenatal diagnosis of fetal chromosomal aneuploidies.

696

Two Genetic Variants in the Methylenetetrahydrofolate Reductase Gene, Maternal Folate and Riboflavin Intake and the Risk of a Congenital Heart Defect. Lydi MJW van Driel,[1] Anna C Verkleij-Hagoort,[1] Robert de Jonge,[2] Andre G Uitterlinden,[3,4] Cock M van Duijn,[4] Regine PM Steegers-Theunissen.[1,4,5,6] (SPON: Eric AP Steegers). *[1]Obstetrics and Gynecology; [2]Clinical Chemistry; [3]Internal Medicine; [4]Epidemiology and Biostatistics; [5]Pediatric Cardiology; [6]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*
Background:Associations between the methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms, involved in the homocysteine pathway, and the occurrence of congenital heart defects (CHD) are contradictory. The frequencies of these functional polymorphisms vary between populations, which may explain some of the discrepancies.
Objective: To determine associations between MTHFR C677T and MTHFR A1298C polymorphisms in mothers, fathers and their children, the periconceptional maternal intake of folate and riboflavin via food and supplements, and the risk of CHD.
Methods:A case-control family study comprising 230 parents with a CHD child and 251 control parents with an unaffected child was conducted in the European population of the Netherlands. Approximately 17 months after the index-pregnancy, standardized questionnaires on periconceptional maternal use of folate and riboflavin supplements and a validated food frequency questionnaire on current maternal dietary intake were filled out. Mothers, fathers and children were genotyped for the MTHFR C677T and A1298C polymorphisms using (Polymerase Chain Reaction)-Restriction Fragment Length Polymorphism. The data were analyzed by logistic regression analysis using the dominant model.
Results:The MTHFR C677T heterozygous and homozygous variant in mothers, fathers and children showed slightly increased odds ratios (OR), varying from OR 1.21 (95% CI 0.84-1.73) to OR 1.27 (95% CI 0.89-1.82), albeit not significantly. However, both the MTHFR A1298C heterozygous

and homozygous variant in fathers and children showed a significantly decreased CHD risk, OR 0.61 (95% CI 0.42-0.88) and 0.66 (95% CI 0.46-0.94), respectively. No significant interactions could be found between these polymorphisms in mothers and children, and maternal folate and riboflavin intake via food and/or supplements.
Conclusions: The MTHFR A1298C polymorphism in fathers and children reduces CHD risk by 40% and 35%, respectively. Interactions between the two MTHFR polymorphisms and maternal folate and riboflavin intake are not involved in the pathogenesis of CHD in the European population investigated.

697

Intercellular Adhesion Molecule-1 Polymorphism in Korean Women with Preterm Delivery. Han-Sung Kwon,[1] Sun-Joo Lee,[1] Ji-Young Lee,[1] In-Suk Sohn,[1] Soo-Nyung Kim,[1] Young-Han Kim.[2] (SPON: Brian J Koos). *[1]Obstetric & Gynecology, Kunkuk University Hospital, Seoul, Republic of Korea; [2]Obstetric & Gynecology, Yonsei University College of Medicine, Seoul, Republic of Korea.*
OBJECTIVES: Increasing evidence shows effects of inflammatory cytokines in the mechanism of infection- and non-infection-induced preterm labor. The cytokines are produced by activated maternal polymorphonuclear leukocytes and monocytes infiltrating the decidual and fetal membranes. Intercellular adhesion molecule-1 (ICAM-1) is an important adhesion molecule that plays a key role in the tight adhesion between leukocytes and vascular endothelium. ICAM-1 is expressed by the human amnion and this expression is elevated with preterm labor and delivery. ICAM-1 shows genetic polymorphisms at codons 469 (K469E). Polymorphisms of this gene was studied in other inflammatory diseases such as rheumatoid arthritis, chronic pancreaitis and so on. The object of this study was to investigate the association between the K469E polymorphism of the ICAM-1 gene and preterm delivery in Korean population.
METHODS: The ICAM-1 K469E polymorphism was genotyped using sequencing analysis in 94 women with preterm delivery and 153 controls who had delivered at least two normal term babies. hs-CRP was assessed in all pateints except in emergent situations.
RESULTS: The distribution of genotype frequencies of the preterm group was significantly different from that of the controls. (KK/KE/EE (%) 36.2/40.4/23.4 vs 43.1/46.4/10.5) The frequency of the K469 allele was significantly lower in preterm group than that in controls. (56.4% vs 67.3%, $p < .05$) Within the preterm group, the K469E allele frequency was lower in the patients with abnormal hs-CRP levels.
CONCLUSION: This preliminary study shows lower frequency of the ICAM-1 K469 allele in Korean women with preterm delivery than in those with term delivery.

698

Prenatal Diagnosis of Fetal Chromosomal Abnormalities by Array-Based Comparative Genomic Hybridization (Array CGH). Ignatia Van den Veyver,[*1,2] Ankita Patel,[2] Sandra Darilek,[2] Marcia Simovich,[2] Carla Suarez,[2] Susan Venable,[2] Xin Yan,[2] Chad Shaw,[2] Patricia Ward,[2] Craig Chinault,[2] James Lupski,[2] Lisa White,[2] Arthur Beaudet,[2] Sau Wai Cheung,[2] Christine Eng.[2] *[1]Obstetrics and Gynecology; [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.*
Objective: A prior validation study on prenatal samples and experience on pediatric clinical samples has shown that array-CGH accurately detects genomic imbalances.
Methods: An array-CGH test designed to optimize detection of clinically significant chromosomal imbalances was offered, in addition to standard karyotype, to women undergoing amniocentesis or CVS. All women received pre-test genetic counseling and provided informed consent. DNA from CVS and amniotic fluid (AF) was prepared after whole genome amplification (WGA), or directly on some CVS, and tested for maternal cell contamination (MCC). Back-up cell cultures were established and standard karyotypes performed for all samples. Parental blood samples were requested to study the origin of copy number variants (CNV) if needed. All positive array-CGH findings were confirmed independently by karyotype, fluorescence in situ hybridization, and/or analysis of parental samples. Data on indications, results and newborn outcomes are collected on an ongoing basis.
Results: Since the validation study (Sahoo et al, 2006), 78 fetal samples were received for clinical array-CGH testing: 63 were AF, 15 were CVS. 37 AF were analyzed on WGA-DNA, and 10 CVS were analyzed on WGA-DNA (3) or direct DNA (7) preparations, yielding results in ≤2 weeks in 60%. Indications were: advanced maternal age (51), abnormal ultrasound (13), abnormality in previous child (10), other positive family history (5), abnormal serum screen result (3), marker identification (2), apparently balanced chromosomal translocation on karyotype (1), prior pregnancy loss (4), parental concern (3) (some had more than one indication). There were 64 normal results and 3 abnormalities (3.8%) (1 trisomy 21, 1 identification of a marker chromosome's origin, 1 deletion at a translocation breakpoint) and 1 pseudomosaic that arose in cell culture. There were 10 CNVs (12.5%), all inherited. There was no MCC.
Conclusions: Array CGH testing yields highly accurate results on CVS and AF in ≤ 2 weeks. Further larger-scale studies should assess whether array CGH can replace karyotyping and FISH for rapid and expanded prenatal detection of chromosomal imbalances.

699

Polymorphisms in the Transcobalamin-2 and Methionine Synthase Reductase Genes, Maternal Vitamin B12 Intake and the Association with Congenital Heart Defects. Anna C Verkleij-Hagoort,[1] Lydi M van Driel,[1] Jan Lindemans,[2] Aaron Isaacs,[3] Willem A Helbing,[4] Andre G Uitterlinden,[2,3,5] Regine P Steegers-Theunissen.[1,3,4,6] (SPON: Eric AP Steegers). *[1]Obstetrics and Gynecology; [2]Clinical Chemistry; [3]Epidemiology and Biostatistics; [4]Pediatric Cardiology; [5]Internal Medicine; [6]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*
Background: Maternal hyperhomocysteinemia increases the risk of having a child with a congenital heart defect (CHD). This might be caused by derangements in vitamin B12 transport and metabolism.
Objective: To investigate associations between polymorphisms in the vitamin B12 transporter gene transcobalamin 2 (TCN2) and the methionine synthase reductase (MTRR) gene, involved in homocysteine remethylation, the maternal vitamin B12 intake and CHDs.
Methods: In the Netherlands a case-control family study was performed in 230 children with a CHD and both parents, and in 251 healthy children and both parents. At 17 months after the index-pregnancy the mothers filled out a general and a food frequency questionnaire covering the current dietary intake and reflecting the periconceptional diet. Blood samples were taken from all families. TCN2 C776G and MTRR A66G genotypes were determined in DNA samples with a Taqman allelic discrimination assay. Non-European families ($n = 95$) were excluded. Data were analysed with transmission disequilibrium tests and logistic regression analyses.
Results: All genotype frequencies were in Hardy-Weinberg equilibrium ($P > 0.05$). Allele transmissions were not significantly distorted. The TCN2 and MTRR genotypes of mothers, fathers and children were not significantly associated with CHD (Table). No significant interactions were observed between these polymorphisms in mothers or children and a low maternal periconceptional vitamin B12 intake and/or no use of a vitamin supplement, and CHD risk.
Conclusions: The TCN2 C776G and MTRR A66G polymorphisms are not associated with CHD. It is unlikely that interactions between these polymorphisms and maternal vitamin B12 intake are involved in the pathogenesis of CHD.

Polymorphisms and CHD risk

			OR (95% CI)	
		Mothers	Fathers	Children
MTRR 66	GG	1.0 (0.6-1.6)	1.5 (0.9-2.6)	1.5 (0.9-2.5)
	AG	0.9 (0.6-1.4)	1.2 (0.7-2.0)	1.2 (0.8-2.0)
	AA		1.0 (Reference)	
TCN2 776	GG	0.8 (0.6-1.3)	1.0 (0.6-1.8)	0.9 (0.5-1.5)
	CG	0.9 (0.5-1.5)	1.1 (0.7-1.6)	0.9 (0.6-1.4)
	CC		1.0 (Reference)	

700

Hyperhomocysteinemia and MTHFR Polymorphisms in Association with Orofacial Clefts and Congenital Heart Defects: A Meta-Analysis. Anna C Verkleij-Hagoort,[1] Johannes B Bliek,[1] Fakhredin Sayed-Tabatabaei,[2,5] Nicolette T Ursem,[1] Eric A Steegers,[*1] Regine P Steegers-Theunissen.[1,4] *[1]Obstetrics and Gynecology; [2]Epidemiology and Biostatistics; [3]Pediatric Cardiology; [4]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [5]Medicines Evaluation Board Agency, The Hague, Netherlands.*
Background: Several studies have reported associations between hyperhomocysteinemia, polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene, and cleft lip with or without cleft palate (CLP) and congenital heart defect (CHD) risk. However, findings have been inconsistent.

SATURDAY

Methods: A meta-analysis was performed of published studies until September 2006 to investigate these associations in both mothers and children. Homocysteine data were provided in two CLP and three CHD studies, and *MTHFR* polymorphisms were reported in ten CLP and eight CHD studies. Data were analyzed using the random effects model in the Cochrane Review Manager.
Results: The pooled odds ratio (OR) of maternal hyperhomocysteinemia was 2.3 (95% CI 0.4-11.9) for CLP, and 4.4 (2.6-7.3) for CHDs. The *MTHFR* C677T polymorphism and CLP showed pooled ORs of 1.2 (0.9-1.5) in mothers and 1.0 (0.9-1.2) in children, whereas these estimates for the *MTHFR* A1298C polymorphism were 1.0 (0.7-1.2) in mothers and 0.9 (0.6-1.2) in children. The *MTHFR* C677T polymorphism in CHD studies demonstrated a pooled OR of 1.0 (0.8-1.3) for mothers and 1.1 (0.9-1.5) for children. Two studies investigating the association between maternal MTHFR A1298C polymorphism and CHDs demonstrated a pooled OR of 1.2 (0.8-1.8). Only one CHD study reported an OR of 1.3 (0.8-2.1) for this polymorphism in children.
Conclusions: This meta-analysis demonstrates that maternal hyperhomocysteinemia is a risk factor for CHDs. The *MTHFR* polymorphisms C677T and A1298C in both the mother and the child are not independently associated with CLP or CHDs. Large studies should be performed to investigate the interactions between maternal hyperhomocysteinemia and other B-vitamin related polymorphisms, and the risk of CLP and CHDs.

701

Teratogens That Cause Autism Also Alter the Expression of Adhesion Molecules. Ujjwal K Rout,[*1] Amy Loflin,[1] Laura Vick,[1] Dirk M Dhossche,[2] John R Gosche.[1] *[1]Department of Surgery, University of Mississippi Medical Center, Jackson, MS, USA; [2]Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS, USA.*
Autism is a severe neurodevelopmental disorder and characterized by pervasive impairments in social interactions, deficits in verbal and non-verbal communication, exploratory and repetitive behavior. The exact cause(s) of autism and timing of autistic development are not known. However, it is becoming evident that both environmental and genetic factors may contribute to autism in children. Recent studies show that autism may result from valproate therapy or maternal alcohol consumption during pregnancy. It is not completely clear however, how exposure to valproate or alcohol during pregnancy may cause autism in the children!
Adhesion molecules such as cadherins and integrins play important role in the brain development, and changes in the expression levels of these adhesion molecules are shown to disturb the normal development of the brain. Therefore, we examined the effects of valproate and alcohol on the expression levels of N-cadherin and β_1 integrin subunit in the neuron-like PC12 cells to understand whether exposure to these compounds during pregnancy may disturb brain development by altering the cellular signaling via these receptors.
Both mRNA and protein levels of these receptor subunits were examined in PC12 cells at different times of culture in absence or presence of valproate or alcohol. Cultures were also supplemented with low or high concentrations of nerve growth factor (NGF) that is known to differentiate PC12 cells. Results show that both valproate and alcohol influence expression levels of N-cadherin and integrin subunit β_1 in PC12 cells in a NGF independent manner. Therefore, maternal exposure to valproate or alcohol during pregnancy may impair the brain development by changing cellular signaling mediated by adhesion molecules.
Supported by a grant from NIH (NIH/NCRR P20 RR017701).

702

Autism Causing Teratogens Alter the Migration of Dissociated Neurons. Ujjwal K Rout.[*] *Department of Surgery and the Center for Psychiatric Neuroscience, University of Mississippi Medical Center, Jackson, MS, USA.*
Autism is a severe neurodevelopmental disorder characterized by pervasive impairments in social interactions, deficits in verbal and non-verbal communication, and exploratory behavior. Exact cause of autism is not known, however both genetic and environmental factors seem to play significant role in the development of Autism Spectrum Disorders (ASD). Recent studies report that exposure to Valproate, thalidomide or alcohol during pregnancy may cause autism in children. The mechanism of this phenomenon is barely known. In the present investigation Boyden Chamber assays were conducted to examine the migratory behavior of dissociated neurons isolated from gestation days 18 rat fetal cerebral cortices. Effects of extra cellular matrix protein laminin, and RGD peptides were tested on the migration of neurons. Both low and higher concentrations of sodium valproate and alcohol (ethanol) on the migratory behavior of neurons were studied in the presence of laminin. Migration of neurons were enhanced by Laminin and reduced by RGD peptide. Alcohol or valproate changed migration of neurons in a dose dependent manner. Results suggest that laminin binding integrin receptors are involved in the migration of dissociated neurons isolated from gestation day 18 rat cerebral cortices. Changes in the migratory behavior of neurons in the presence of valproate or alcohol *in vitro* indicate that maternal exposure to these teratogens during second trimester of pregnancy may alter the positioning of neurons causing ASD.
Supported by a grant from NIH (NIH/NCRR P20 RR017701).

703

Chronic Exposure to Increasing Concentrations of Alcohol during Pregnancy Changes Maternal, Fetal and Placental Weights. Ujjwal K Rout.[*] *Department of Sugery and The Center for Psychiatric Neuroscience, University of Mississippi Medical Center, Jackson, MS, USA.*
Maternal exposure to alcohol results in wide range of abnormalities in the offspring. Studies to examine the effects of alcohol on the developing fetus report both genotype and time dependent changes in the fetal weights. Placental weight is also reported to change due to the gestational alcohol-exposure. These studies however used constant and higher doses of maternal alcohol exposure on the weight of organs which might not allow the mothers and fetuses to adapt to the alcohol's toxic effects. Therefore in the present investigation, increasing concentrations of alcohol in the isocaloric protein fortified diet was used to determine if gradual increase in the alcohol would prevent the adverse effects of alcohol. Long-Evans rats were given protein fortified diet with 2.2% alcohol during gestation days 6 and 7, 4.5% alcohol during gestation days 8 to 10 followed by 6.7% alcohol until sacrifice. Control animals obtained the diet with no alcohol. Appropriate amounts of Maltose dextran was added to ensure the control and alcohol diet to be isocaloric. On gestation days 18 or 20 morning control and alcohol-exposed mothers were anesthetized with isoflurane, weighed and dissected to obtain the fetuses and placentas. Fetal and placental weights were determined. ANOVA test was used to determine changes in the weights due to gestational alcohol. Significant drops in mothers and fetal weights due to alcohol exposure were observed. Placental weights, in contrary, were increased in the alcohol exposed groups. Results suggest that exposure to increasing concentrations of alcohol during pregnancy starting even with a lower dose in the first trimester may not prevent alcohol's toxic effects on the mother and fetus.
Supported by a grant from NIH (NIH/NCRR P20 RR017701).

704

Array-CGH Analysis of Cell-Free Fetal DNA in Amniotic Fluid Detects Human Aneuploidies. Olav Lapaire,[1] Kirby L Johnson,[2] XinYan Lu,[3] Wolfgang Holzgreve,[*1] Diana W Bianchi.[2] *[1]Department of Obstetrics and Gynecology, University of Basel, Basel, Switzerland; [2]Department of Pediatrics, Tufts-New England Medical Center, Boston, MA, USA; [3]Spectral Genomics, Inc, Houston, TX, USA.*
Background. Previously we showed that comparative genomic hybridization (CGH) analysis of cell-free fetal (cff) DNA isolated from amniotic fluid (AF) allows for prenatal molecular karyotyping and fetal gender identification (Am J Hum Genet 75:485-91). Subsequent technical advances have increased the yield of extracted cffDNA to permit CGH analysis of ≤10 mL of AF (Clin Chem 52:156-7). Here we aimed to determine whether a variety of fetal aneuploidies can be identified thorough the analysis of cffDNA from AF by array-CGH.
Methods. CffDNA was extracted from ≤10 mL of residual AF supernatant using AVL buffer and maxi columns (Qiagen, Valencia, CA). In all cases, test cffDNA and reference DNA were labeled with different fluorescent dyes and applied to Spectral Constitutional Chip™ 2.0. The following fetal abnormalities were analyzed: trisomies 13 (n=1), 18 (n=3), and 21 (n=2), as well as cases of mosaicism (47,XX,+9[18]/46,XX[2]) and triploidy (69,XXY).
Results. CffDNA isolated from aneuploid fetuses showed increased hybridization signals on the majority of the affected chromosome markers compared to euploid reference DNA. Aneuploid fetuses were correctly identified in all cases.
Conclusions. These results indicate that cffDNA extracted from ≤10 mL can be analyzed using array-CGH to correctly identify human autosomal and sex chromosome aneuploidies. In conjunction with previous work, we show that this technology allows for rapid screening of AF samples for whole chromosomal changes without interfering with current standard of care and may augment standard karyotyping techniques by providing additional molecular information.

705

Echocardiographic Indications To Detect Chromosome 22 Microdeletions through Invasive Prenatal Procedures. M Infantino,[1] B Gentilin,[1] E Brandolisio,[2] A Colli,[3] L Mauri,[3] L Mandia,[1] A Kustermann,[1] Irene Cetin.*[1] *[1]Dept for the Health of Women, Neonate and Child, IRCCS Foundation PoMaRE, University of Milan, Milan, Italy; [2]Medical Genetics Laboratory, IRCCS Foundation PoMaRE, University of Milan, Milan, Italy; [3]Dept of Paediadric Cardiology, IRCCS Foundation PoMaRE, University of Milan, Milan, Italy.*
Introduction: Chromosome 22 microdeletion syndrome (DEL22) is associated with a clinical picture once identified with the acronym CATCH 22 (Cardiac and facial Anomalies, Thymic hypoplasia, Cleft palate, Hypocalcaemia and deletion 22q11). The incidence of this syndrome is of 1: 4000 born alive and is a frequent genetic cause of Congenital Heart Disease (CHD), second most frequent after Down's syndrome. This condition is often associated with a conotruncal cardiac defect but at present, there are no guidelines and/or clear indications to search for this deletion in utero. **Objectives:** to evaluate the correlation between echocardiographic indications to search for DEL 22 syndrome and molecular and pathology results. **Methods:** We retrospectively reviewed 31 cases where prenatal diagnosis of CHD led to prenatal karyotyping and FISH analysis of Chromosome 22q11.2. **Results:** Deletion of Chromosome 22 was present in 5/31 (16%) fetuses. All 5 DEL 22 positive fetuses had conotruncal defects, 3/3 fetuses with aortic arch defects and 2/9 fetuses with Fallot's Tetralogy, representing a 25% (5/20) of the conotruncal defects identified in this cohort. Facial dysmorphism and/or thymic hypoplasia was reported in all pathology examinations of the cases with deletion. **Conclusions:** This study confirms previous evidence of the known association between conotruncal cardiac defects and DEL 22. Moreover, our data suggest that additional features such as mycrognathya and/or thymic hypoplasia should be present to support a more selective search for the microdeletion of Chromosome 22.

706

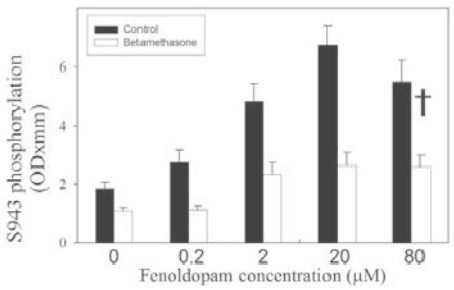
Proteomics of Non-Smoker and Smoker Term Placentas. Frederick Naftolin,*[1] Seyed Monemian,[1] John Fitzgerald,[1] Pevsner H Pevsner.[1] *[1]Obstetrics and Gynecology, NYU School of Medicine, New York, NY, USA; [2]Pharmacology, NYU School of Medicine, New York, NY, USA; [3]Pharmacology, NYU School of Medicine, New York, NY, USA; [4]Pharmacology, NYU School of Medicine, New York, NY, USA.*
Term placenta, 1micron tissue slices, of non-smokers and smokers were examined with matrix assisted laser desorption ionization mass spectrometry, MALDI MS, and MALDI MS/MS. Trypsin 1:10,000 w/w of protein and matrix, (alpha cyano 4 hydroxy cinnamic acid, 10mg/cc) 100:1 w/w of protein were applied directly to the tissue slices. The tissue was frozen, cut on a cryomicrotome, and the sections applied to conductive metal plates with mirror image sections applied to glass slides for MALDI and histologic examination, respectively. There were significant differences in the peptides identified by the tryptic digestion. These peptides were subjected to MALDI MS/MS in a collision chamber and dissociated with helium gas. The mass/charge (m/z) spectra were subjected to bioinformatics via Mascot Ion software interogation of the NCBI database. Peptide sequencing and protein identification including increased levels of metallothionien 2 isoforms in placentas of smokers compared to non-smokers was obtained with this technique. The study demonstrates proof of principle for MALDI MS/MS identification of proteins directly from placental tissue, and outlines the specificdetails of the technique for investigation of the placenta.

707

Novel MEMS Application for Capture and Analysis of Circulating Fetal Nucleated RBCs for Definitive First Trimester Non-Invasive Prenatal Genetic Diagnosis of Aneuploidies. Farideh Z Bischoff,[1] Pavel Tsinberg,[2] Joe Leigh Simpson,*[1] Ronald Wapner,[3] Philip Cotter.[2] *[1]Baylor College of Medicine, Houston, TX, USA; [2]Biocept Inc, San Diego, CA, USA; [3]New York-Presbyterian Hospital, New York, NY, USA.*
INTRODUCTION Definitive recovery of fetal cells for first trimester genetic diagnosis currently requires chorionic villus sampling (CVS). Non-invasive approaches for aneuploidy detection are widely applied, approximately 80-90% detection; however, these approaches are not alone definitive. Definitive non-invasive approaches have focused on the isolation of fetal nucleated erythrocytes or trophoblasts. Fetal nucleated red blood cells (fnRBCs) are recovered in maternal blood as early as six weeks gestation. However, enrichment and molecular cytogenetic analysis of fnRBCs from maternal blood have been unreliable to date. We believe the problem can be surmounted by MEMS (microelectromechanical system), whose principle is to capture cells based on attachment chemistry and fluid dynamics. The objective of our study was to determine feasibility using MEMS for targeted cell enrichment and analysis of first trimester fnRBCs from blood samples.
METHODS A male cord blood specimen from a first trimester (7 wks) termination was spiked into non-pregnant blood (n=6) at a concentration of 200 fetal cells per 10 ml, performed in triplicate. Following ficoll 1077 separation, recovered cell suspensions were run on glycophorin-A coated MEMS channels. After capture, cells were subjected to direct FISH analysis within MEMS channels for detection of X- and Y-chromosomes (Vysis Inc.). As a control, cord blood was processed by H & E staining to determine frequency of fnRBCs amenable to capture.
RESULTS In the cord blood, 10% of total fetal cells were fnRBC stain positive. Thus, a capture rate of approximately 20±5 glycophorin-A positive fetal cells would be expected in the spikes. In these experiments, fetal XY-cells were recovered in all six, in numbers ranging from 16 to 68 XY-cells per channel. Background frequencies of female (XX) cells in the recovered samples were 350 to 10,000 cells.
CONCLUSIONS Our novel enrichment strategy utilizing MEMS shows feasibility of isolating fnRBCs with a high degree of purity from peripheral blood. This novel technology can be applied for early prenatal aneuploidy detection.

708

Antenatal Betamethasone Exposure at 80 Days Gestation Alters the Regulation of Renal Na-K ATPase in Adult Sheep Offspring. Jing Wang, Jie Zhang, Angela G Massmann, Jorge P Figueroa.* *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*
OBJECTIVE: In rats and sheep exposure to GC in the perinatal period induces hypertension in adult life. The activity of the Na-K ATPase pump is considered to be an important mechanism in the regulation of sodium reabsorption. Phosphorylation (p) of the α1 subunit of Na-K ATPase at Ser 943 by PKA regulate pump activity and membrane translocation. Alterations in sodium handling are associated with hypertension both in humans and in animal models. The aim of the study was to determine kidney proximal tubules (PT) Na-K ATPase phosphorylation status under basal conditions and following dopamine D1 receptor agonist stimulation.
METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (V) 24 h's apart at 80 days of gestational age and allowed to deliver at term. At 1.5 yr of age the offspring were euthanized and kidney proximal tubules were prepared by collagenase digestion and Percoll gradient separation. PT were treated with increasing doses of the D1 dopamine agonist fenoldopam (0.2, 2, 20 and 80 μM) for 10 min. The reaction was stopped by the addition of 0.3 ml cold lysis buffer. ATPase phosphorylation at pSer-943 of the α1 subunit and native α1 subunit abundance were evaluated by western blot. Data are expressed as Mean±SEM and were analyzed by Two way ANOVA.
RESULTS: No differences in α1 subunit abundance were observed between the two groups. Dose dependent phosphorylation of Ser-943 was observed in both groups (†). Although basal p of α1 Na-K ATPase Ser-943 levels were not different, in response to D1 agonist, pSer-943 abundance was significantly lower in PT of BM exposed sheep (Figure, $p<0.05$).
CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation decreases pSer-943 in response to dopamine stimulation suggesting an impairment in the kidneys ability to regulate sodium reabsorption. A decrease in dopamine natriuretic effect is associated with hypertension in humans and in animal models of hypertension. HL 68728; HD P01 HD04784.



SATURDAY

709

Localization and Gestation-Dependent Pattern of CRF-Receptors (R1, R2) Expression in Ovine Fetal Distal Colon. Jayaraman Lakshmanan, Guong Liu, Noboru Oyachi, Michael Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: As in stress-induced adult defecation, in utero meconium passage requires participation of CRF and CRF receptors in fetal colon motility responses. CRF mediates its biological actions through CRF-receptor type 1 (CRF-R1; colon motility stimulatory) and CRF-receptor type 2 (CRF-R2; colon motility inhibitory). We sought to localize the CRF-R1 and R2 receptors in ovine fetal distal colon to address their contribution to stress-induced meconium passage.

Method:

The distal colon was dissected from ovine fetuses (n=4 for each age) at very preterm (VPT: 118-120 d), preterm (PT: 130-132 d), near term (NT: 140-142 d) and term (T:146-147 d) and contents flushed with ice-cold phosphate buffered saline. Segments of 4 cm were prepared and colonic rings (3-4 mm) cut at both end of segments, fixed in Bouin's solution and paraffin embedded. Sections were processed for immunostaining with rabbit polyclonal antibodies specific to CRF-R1 (generously provided by J. Rose) and CRF-R2 (1:200-1:400 Abcam) by avidin-biotin-peroxidase system. Double immunofluorescence and laser confocal analyses were performed to ascertain the neuronal localization of CRF-receptors. Percent of myenteric and submucosal ganglia immunostained were counted and intensity of immunostaining in circular smooth muscle layer was analyzed using Image Pro-plus software.

Results: CRF-R1 and CRF-R2 antibody strongly immunostained longitudinal and circular smooth muscle layers in the distal colonic segments at all four gestational ages. A marked decrease ($p<0.0001$) in CRF-R2 immunostaining occurred in circular muscle at term compared to very preterm (IOD: 9.3±1.4 vs 44.2±4.5). CRF-R1 though not CRF-R2 stained neuronal somas in the myenteric and submucosal ganglia. Percent of ganglia expressing CRF-R1 receptor significantly and markedly increased at term in both myenteric (VPT: 16.2±0.7, PT: 23.2±0.6, NT: 27.0±0.5 and T: 64.7±0.6 %) and submucosal ganglia (VPT: 6.1±0.5, PT: 14.3±0.3, NT: 16.5±0.3 and T: 31.7±0.8 %).

Conclusion: CRF-R1 and R2 receptors are expressed during fetal periods of development in ovine fetal distal colon. Increased early gestation CRF-R2 expression may inhibit colonic motility, while reduced late gestation CRF-R2 expression combined with increased ganglia CRF-R1 expression may potentiate stress-induced colonic motility and meconium passage.

710

Collagen Gel Contraction by Vascular Smooth Muscle Cells from the Human Placenta: Regulation of Angiotensin II on Placental Vessel Cell Contractility. Clint Benoit,[1] Yang Gu,[1] Yanping Zhang,[1] J Steven Alexander,[2] David F Lewis,[1] Yuping Wang.*[1,2] *[1]Obstetrics and Gynecology; [2]Molecular and Cellular Physiology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: Angiotensin I (Ang I) and II are present in the placenta and the uteroplacental unit possesses all components of the Renin-angiotensin system (RAS) necessary for their generation. Altered placental Ang II receptor-1 (AT1) expression has been considered playing a role in enhanced oxidative stress and increased vasoconstriction in pregnancy-complicated disorders such as preeclampsia and IUGR. However, the pathways of Ang II generation and its receptors AT1 and AT2 regulation in placental vessel smooth muscle cells (VSMCs) have not been studied. In the present work, we used a collagen gel contraction model to investigate the role of Ang II and its receptor regulation in placental VSMC contractility.

Methods: VSMCs were generated from tertiary chorionic plate vessels from placentas delivered by normal pregnant women. Type-1 collagen was prepared from rat-tail tendons. Placental VSMC/type-1 collagen gels were incubated with placental conditioned medium (CM). Captopril (an ACE inhibitor) and chymostatin (a chymase inhibitor) were used to study effects of ACE and non-ACE on Ang II generation. Losartan (an AT1 receptor blocker) and PD123319 (an AT2 receptor blocker) were used to study specific AT1 and AT2 receptor regulation, respectively. Protein expressions for AT1 and AT2 were determined by Western blot analysis.

Results: 1) chymostatin but not captopril significantly blocked placental VSMC/collagen gel contraction induced by placental CM, $p<0.01$; 2) PD123319, but not losartan, also markedly attenuated placental VSMC/collagen gel contraction, $p<0.01$. The inhibitory effects are in a dose-dependent manner for both chymostatin and PD123319; and 3) AT1 protein was strongly expressed in VSMCs, but the expression level was not affected when cells were stimulated with placental CM. In contrast, AT2 protein was weakly expressed in control cells, but upregulated upon stimulation.

Conclusions: Our results suggest that chymase, a non-ACE Ang II generating enzyme, rather than ACE may play a dominant role in controlling Ang II generation in the human placenta. AT2 receptor may be more responsible for regulating Ang II induced contractility in placental VSMCs. These results provide new insights into control of Ang II generation and Ang II receptor regulation in the placental vasculature.

711

Uterine Natural Killer Cell Regulation of Trophoblast Invasion. Gendie E Lash,[1] Harry A Otun,[1] Kathryn Percival,[1] Barbara A Innes,[1] Judith N Bulmer,[2] Roger F Searle,[3] Stephen C Robson.*[1] *[1]SARS, Newcastle University, Newcastle, United Kingdom; [2]CALS, Newcastle University, Newcastle, United Kingdom; [3]MED, Newcastle University, Newcastle, United Kingdom.*

Background: Understanding the mechanisms underlying the regulation of extravillous trophoblast (EVT) invasion is central to understanding human placentation. Uterine natural killer (uNK) cells have been proposed to play a role in this process by secretion of various cytokines and growth factors. We have previously demonstrated that TGFβ1, TNFα and IFNγ inhibit EVT invasion through a mechanism partially dependent on altered protease activity.

Hypothesis: uNK cells inhibit EVT invasion in part by secretion of TGFβ1, TNFα and IFNγ and inhibition of protease activity.

Methods: Decidual and placental samples were obtained with informed consent from women undergoing pregnancy termination (8-10 [n=10] and 12-14 [n=8] weeks gestational age). After enzymatic disaggregation CD56+ uNK cells were positively selected (>95% purity) using an immunomagnetic technique. uNK cells were cultured for 48 hours and supernatants (SNs) harvested. Invasion assays were performed with placental explants (8-10 weeks gestation) on Matrigel coated 8μm inserts in the presence of uNK cell SNs ± anti-TGFβ1, anti-TNFα or anti-IFNγ. After 6 days culture medium was harvested for analysis of MMP2, MMP9 and uPA protein levels by gelatin or casein/plasminogen zymography. Inserts were stained, mounted on glass slides and the whole of the filter counted. Data are shown as invasion index relative to control for each experiment; mean±SEM.

Results: uNK cell SNs from 8-10 weeks gestation had no effect on EVT invasion. uNK cell SNs from 12-14 weeks gestation stimulated EVT invasion (control 1.0±0.0, uNK 2.7±0.5; P=0.04). Each of the neutralising antibodies partially attenuated the effect of the uNK cell SNs on EVT invasion (anti-TGFβ1 2.2±0.8, anti-TNFα 1.7±0.6, anti-IFNγ 1.3±0.5) with no statistical difference compared with controls. There was no difference in MMP2 or uPA levels under any of the conditions tested. MMP9 levels were increased in the presence of uNK cell SNs.

Conclusions: Although uNK cell SNs from 8-10 week samples had no effect on EVT invasion SNs from 12-14 week samples stimulated EVT invasion via a mechanism partially dependent on increased MMP9 levels. uNK derived TGFβ1, TNFα and IFNγ do not appear to be significantly involved in this process. uNK cells may play a role in maintenance of EVT invasion *in vivo*.

712

Temporal and Cell Specific Regulation of the RNA Binding Protein, VICKZ, in Normal and Pathological Placentae. Tal Imbar,[1] Gilad Vainer,[2] Debra Goldman-Wohl,[1] Caryn Grenfield,[1] Joel K Yisraeli,[2] Drorith Hochner-Celnikier.[1] (SPON: Simcha Yagel). *[1]Obstetrics and Gynecology, Hadassah University Medical Center, Jerusalem, Israel; [2]Anatomy and Cell Biology, Hadassah University Medical Center, Jerusalem, Israel.*

OBJECTIVE: The family of VICKZ RNA binding proteins can localize RNAs to the leading edge of migrating cells. VICKZ expression is associated with cell invasion and metastasis in several types of neoplasms. Its expression in normal human development and placentation is largely unknown. Our objective was to determine the expression pattern of VICKZ proteins in normal and abnormal placentation.

METHODS: The expression of VICKZ was examined using immunohistochemistry with a pan VICKZ antibody. Serial sections were examined with a cytokeratin antibody to identify trophoblasts. Normal first, second and third trimester placental tissue sections were studied. Placental tissue sections taken from pathological conditions including: complete and partial hydatidiform mole, preeclampsia, placenta accreta following a previous cesarean section, placenta accreta with no previous uterine injury and ectopic pregnancies were examined. A speciman from a normal non-pregnant uterus was included in the study.

RESULTS: VICKZ was found to be highly expressed in extravillous trophoblasts of first trimester placenta. Its expression was down regulated in third trimester. Similar findings were observed in pathological sections

of first trimester (ectopic, complete and partial mole) where expression was high in extravillous trophoblasts. In third trimester pathological samples (preeclampsia and accreta) expression was down regulated. Cytokeratin immunohistochemistry confirmed the presence of trophoblsts in these samples. We also observed expression of VICKZ in the glandular epithelium of the non-pregnant uterus.

CONCLUSION: The temporal and cell specific regulation of VICKZ expression in the placenta and its expression in first trimester placenta, suggests that VICKZ expression may play a role in regulating invasion of extravillous trophoblasts. We conclude that VICKZ may have an important role in the process of normal as well as abnormal placentation.

713

Heparanase Is Activated at the Leading Front of Cytotrophoblast Invasion at the Human Feto-Maternal Interface. Ronit Haimov-Kochman,[*1] Shira Natanson-Yaron,[1] Caryn Greenfield,[1] Debra Goldman-Wohl,[1] Arye Hurwitz,[1] Israel Vlodavsky,[2] Simcha Yagel.[*1] *[1]Obstetrics and Gynecology, Hadassah Hebrew University Medical Center, Jerusalem, Israel; [2]Vascular Biology and Cancer Research, The Bruce Rappaport Faculty of Medicine, Haifa, Israel.*

Objective Heparanase, a mammalian endoglycosidase, capable of depolymerizing heparan sulfate (HS) chains, is abundant in the placenta. Cytotrophoblasts (CTBs) produce two heparanase isoforms. The full-length 65kDa proheparanase undergoes proteolytic processing yielding an active 50 kDa enzyme heterodimer. Recently, the protease that cleaves proheparanase has been identified as cathepsin L that exists in both lysosomal and secreted forms. We sought to determine the distribution of heparanase isoenzymes and cathepsin L in the human implantation site.

Methods Heparanase isoforms and cathepsin L were immunolocalized on cultured 1st trimester placental villi and basal plate of 2nd trimester placenta. The heparanase proenzyme and the active isoform were detected using anti-linker (anti-CTKL) and 733pAb, respectively. An affinity-purified cathepsin L IgG (R&D Systems, Inc) raised against the recombinant protein, with less than 1% cross-reactivity with other cathepsins was used to immunodetect cathepsin L.

Results HLA-G-positive CTB columns of 2nd trimester villous placenta expressed both active heparanase and cathepsin L. Likewise, HLA-G-positive CTBs of placental villi invading the Matrigel were immuno-reactive with both active heparanase and cathepsin L but lack the expression of proheparanase. Proheparanase was exclusively detected at the villous stroma.

The human implantation site showed reactivity with both heparanase isoenzymes and cathepsin L. Interestingly, differential expression of heparanase isoforms was detected along the invasive front of CTBs in the decidua. Active heparanase and cathepsin L were co-localized at HLA-G-positive extravillous CTBs interstitially invading the decidua whereas CTBs lingering behind expressed only the relatively inactive isoform of heparanase.

Conclusion The proteolytic activation of heparanase by cathepsin L provides an attractive means by which heparanase-mediated cleavage of decidual HS is regulated and invasion of CTSs enhanced. Our data show that the most leading fronts of CTB invasion express cathepsin L that potentially depletes the reservoir of tissue proheparanase and enhances the activation of heparanase.

714

Oxygen Modulates Cell Column Formation and Invasion of First Trimester Cytotrophoblasts into Amnionic Mesenchyme. Eline Poliard,[1] Mamed Kadyrov,[2] Louis Peeters,[*1] Berthold Huppertz.[3] *[1]Ob & Gyn, Univ Hosp Maastricht, Netherlands; [2]Anatomy II, Univ Hosp RWTH Aachen, Germany; [3]Cell Biol, Hist & Embr, Med Univ Graz, Austria.*

Objectives: During invasion into the maternal decidua, cytotrophoblasts (CT) undergo a phenotypical shift possibly modulated by oxygen. We used a double tissue culture system with first trimester villous explants on term amnion to study cell column (CC) formation, phenotypical changes, apoptosis and proliferation of CTs in different oxygen conditions during invasion.

Methods: First trimester chorionic villi (5 to 9 weeks gestation, n=16) were collected after surgical termination of pregnancies and fetal membranes were obtained from elective term C-sections (n=6). Villous explants were placed on the mesenchymal side of amnion pieces and cultured in DMEM/F12. Explants were exposed to 20% O_2 or 2.5% O_2 for up to 8 days. Histology (HE) and immunohistochemistry (cytokeratin 7 and 18, Mib-1, M30, GLUT1, MMP3, CA9, Hif 2α) were performed.

Results: After 3 days, invading CTs had crossed the spongy layer and reached the compact layer of the amnion. In 2.5% O_2 CT showed more proliferation (Mib-1) and less apoptosis (M30). In both oxygen conditions, apoptosis of CTs was more pronounced in villi than in invading CTs. In 20% O_2, more CCs were generated (# CC) which invaded deeper into the mesenchyme (depth CC). Meanwhile, the size of the CCs (size CC) was larger compared to 2.5% O_2. In both conditions, we noted Glut 1 expression in the apical microvilli of the syncytiotrophoblast, in the CTs and in the amnion epithelium. Hif 2α expression was most dominant in the syncytiotrophoblast, villous stroma and in invading CTs, but negative in the CCs, whereas CA9 was only expressed in the villous stroma.

The table contains descriptive statistics and p values.

Conclusions: This model of CT invasion nicely mimics the in vivo situation of invasion. The differential CC formation of early first trimester villi is consistent with a direct effect of oxygen on the invasion of CTs in that period of pregnancy. Whether late first trimester explants respond similarly to different oxygen conditions remains to be determined.

	Mib-1 (%)	M30 (%)	# CC (/300µm²)	depth CC (µm)	size CC (µm²)
20 % O_2	13.9 ± 2.2	43.4 ± 3.4	6 ± 1.1	57.7 ± 8.5	5458.8 ± 986.2
2,5 % O_2	42.4 ± 4.5	24.7 ± 2.8	3 ± 0.7	40.4 ± 3.8	1964.6 ± 279.3
p- value	<0.0001	0.0001	0.0279	0.0333	0.0177

We compared the observations in the 2 oxygen conditions by either the Mann-Whitney-U Test or unpaired Student-*t*-test. Values are expressed as mean ± SEM.

715

Characterization of Numb Expression in the Human Placenta. Mariha Hadier,[1] Qing Qiu,[1] Mahmud Bani-Yaghoub,[2] Benjamin K Tsang,[*1] Andree Gruslin.[*1,3] *[1]Hormones, Growth and Development Program, Ottawa Health Research Institute, Ottawa, ON, Canada; [2]Neurogenesis & Brain Repair Group, NRC, Ottawa, ON, Canada; [3]Dept. of OBGY, Ottawa Hospital, Ottawa, ON, Canada.*

Introduction: Numb is a multifunctional protein involved in cell differentiation and proliferation associated with neurogenesis. This protein has four alternative splicing isoforms with distinct functions. Numb null mice have incidentally been noted to display defects in placental formation, suggesting Numb may play a role in placental development. However, the expression of Numb in the human placenta or on the presence of its isoforms are unknown

Objective: To examine the expression and localization of Numb in human EVT cell line and placental tissue**.**

Methods: Numb transcripts were examined by RT-PCR with three sets of primers, in which the amplified fragment included (1) the sequence coding N-terminal phophotyrosine binding (PTB) domain region only, (2) a proline–rich C-terminal region (PRR) only and, (3) both PTB and PRR respectively in HTR8/SVneo and human placenta. Numb protein expression were also assessed with Western blot analysis. The localization of Numb protein in the human placenta was examined with immunohistochemistry(IHC). Vimentin and cytokeratin proteins were used as cell markers for decidual and EVT at the maternal-fetal interface respectively.

Results: Two fragments with sizes of 316 and 283 bp were detected by PCR using a primer located in nucleotides coding for PTB domain. The fragments of 428 and 284 bp were amplified with a primer designed from the PRR region. The PCR analysis with primer designed to amplify the fragments including both PTB domain and PRR regions showed 1188, 1155, 1044 and 1011 bp products, confirming that all 4 Numb isoforms are expressed in the EVT cell line and human placenta. Western blot analysis revealed the presence of 71 and 72 kDa numb proteins in both the cell line and placental tissue. Numb immunosignals in scatter cytotrophoblasts and stromal cells were observed in placental villi at the 1st and 3rd trimester with IHC. At the maternal-fetal interface at 1st trimester, both decidual and EVT displayed strong Numb immunoreactivity.

Conclusion: We have demonstrated for the first time, that the four numb isoforms are present in a human EVT cell line and placentae. Numb protein is localized to cytotrophoblasts and EVT. These results suggest that Numb may be involved in placental development, more specifically in trophoblast invasion.

716

ADAM Expression in Human Trophoblast Cells under Hypoxic Conditions. Maureen Lee, Oscar L Buzzio, Vanessa R Sapoznik, Richard E Leach.* *Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, USA.*

Background: Heparin-binding EGF-like growth factor (HB-EGF) is normally expressed in the placenta and found to be deficient in preeclampsia (Lancet 360, 1215-1219; 2002). HB-EGF has prosurvial, antiapoptotic actions that are initiated by metalloprotease (MP) cleavage of HB-EGF to the secreted form. General MP inhibition with GM6001 prevents the cleavage of HB-EGF and subsequent transactivation of both HER1 and HER4 required for the downstream signaling of HB-EGF in the hypoxic cell context of early gestation (Development 133:751-759; 2006). Although various ADAMs (a disintegrin and metalloproteinase) have been shown to mediate HB-EGF cleavage in other cell types under hypoxic conditions, it is currently unknown which ADAMs are expressed in trophoblast cells.

Objective: Elucidate specific ADAM expression in an immortalized human cytotrophoblast cell line, HTR-8/SVneo (HTR) cells.

Methods: A search of the UniGene Data Base reveals ADAM 9, 10, 12, 15 and 17 to be highly represented in normalized human placental EST libraries, consistent with individual ADAM gene expression (Genomics 72:34-42; 2001). HTR cells were exposed to hypoxia (2% O_2) or normoxia (20 % O_2) for 8 h and ADAMs 9, 10, 12, 15 and 17 expression was determined by Western blot analyses of membrane cell extracts.

Results: Protein analyses indicate the presence of ADAM9 (70 and 100 KDa), ADAM12 (a double band around 92 KDa), ADAM15 (90 KDa) and ADAM17 (150 KDa) in membrane cell extracts of HTR cells, and no difference in the expression of these proteins between 2% and 20% O_2. ADAM10 is not expressed in HTR cells under 2% and 20% O_2 conditions.

Conclusions: To our knowledge, this is the first time that the presence of ADAMs is assessed in a trophoblast cell line. We found no difference in the protein expression levels of four ADAMs between 2% and 20% O_2. These findings suggest that HB-EGF cleavage to the secreted form under hypoxic condtions is unlikely due to ADAM activity. This will be confirmed by gel zymography to determine differential activity between hypoxic and normoxic conditions. Supported by National Institutes of Health grant to REL (HD37500).

717

Effect of Decidual $CD8^+$ T Lymphocytes on Protease Production by Placental Explants. Leandro G Oliveira,[1] Paula J Scaife,[1] Gendie E Lash,[1] Harry A Otun,[1] Barbara A Innes,[1] Katsuhiko Naruse,[1] Roger F Searle,[2] Stephen C Robson,*[1] Judith N Bulmer.[3] *[1]SARS, Newcastle University, Newcastle upon Tyne, United Kingdom; [2]MED, Newcastle University, Newcastle upon Tyne, United Kingdom; [3]CALS, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Background: Invasion of uterine decidua, myometrium and spiral arteries by extravillous trophoblast (EVT) is crucial for healthy pregnancy and decidual leukocytes may be involved in control of this process. T cells account for fewer than 20% of leukocytes in first trimester human decidua, although they account for a higher proportion in the third trimester. We have previously demonstrated that supernatants produced by decidual $CD8^+$ lymphocytes (8-12 weeks gestational age) stimulate invasion of EVT from first trimester placental explants. The mechanism underlying this stimulation of invasion is not known.

Hypothesis: Supernatants produced by decidual $CD8^+$ T lymphocytes stimulate EVT invasion via an increase in protease activity.

Methods: Decidua was obtained with informed consent from women undergoing termination of healthy pregnancy (8-10 and 12-14 weeks gestational age, n=10 both groups). After enzymatic disaggregation $CD8^+$ T lymphocytes were positively selected (>95% $CD8^+$) using an immunomagnetic technique. Isolated cells were incubated with phytohaemagglutinin (PHA)-P (10mg/ml) and supernatants harvested after 24 hours. Decidual $CD8^+$ lymphocyte supernatants (33% vol/vol in DMEM-F12 containing 10% FBS) were cultured with placental tissue explants of the same gestational age on Matrigel in flat bottom wells. After 6 days of incubation supernatants were harvested from the placental explants and MMP-2, MMP-9 and uPA activities were determined by gelatin or casein/plasminogen gel zymography.

Results: Although EVT invasion was increased by decidual $CD8^+$ T lymphocyte supernatants, levels of MMP-2 and MMP-9 activity were decreased in explants cultured with decidual $CD8^+$ T lymphocyte supernatants when compared with the PHA-P control in the 8-10 weeks gestation group (P=0.047 and P=0.047, respectively). A trend towards the same effect was seen for MMP-9 at 12-14 weeks gestation, but was not statistically significant (P=0.09). uPA activity was not affected by culture in decidual $CD8^+$ supernatants in either gestational age group.

Conclusion: Decidual $CD8^+$ T lymphocytes can regulate the invasive capacity of EVT *in vitro*. We speculate that this regulatory effect involves mechanisms independent of protease activity.

718

Identification of Estrogen-Regulated Human Endogenous Retroviral (HERV) Genes in a Trophoblast Cell Line. Jun Sugimoto,[1,2] Makiko Sugimoto,[1] Danny J Schust.*[1] *[1]Obstetrics, Gynecology and Women's Health, University of Missouri-Columbia School of Medicine, Columbia, MO, USA; [2]Molecular Biology, Ryukyu University, Naha, Japan.*

HYPOTHESIS: Reproductive steroid exposure regulates trophoblast HERV expression. Retroviral sequences comprise approximately 8-10% of the human genome. The vast majority of sequences encoded by these human endogenous retroviruses (HERVs) are not expressed. Of those very few that are, some have been associated with physiologic function; others with disease. The placenta is unique in its expression of a small number of functional retroviral sequences that encode expressed proteins. Syncytin is one example. It is expressed in at high levels in cytotrophoblast cells and has a role in syncytialization to syncytiotrophoblast. Human pregnancy is marked by an early and sustained rise in reproductive hormone secretion. In this study, we use a cell line representing cytotrophoblast (JEG3) to examine the effects of the reproductive steroid, estradiol, on well-described (HERV-W, HERV-F) and novel (HERV-E) placental HERV families.

METHODS: HERV- expression was assessed using a novel sequence-based RT-PCR method that detects both qualitative and quantitative characteristics of alterations in HERV family gene transcription. Amplification of DNA from estradiol exposed (10^{-6} M, 10^{-7} M, and 10^{-8} M; 0-48 hours) and non-exposed JEG3 cells was subjected to amplification by degenerate HERV family-specific primers. Sequencing of amplified products allowed quantification of the proportion of products that coded for specific HERV family subtypes.

RESULTS: HERV-W subtype expression (including expression of syncytin) was markedly affected by estradiol exposure, while HERF-FRD expression (syncytin 2) was unaffected by treatment. HERV-E products are newly described to be expressed in the placenta; one interesting subtype (localized to chromosome 17) exhibited transcriptional upregulation with estradiol exposure.

CONCLUSIONS: Reproductive steroid exposures typical of the human menstrual cycle and human pregnancy modulate the expression of functional and novel, potentially functional endogenous retroviral genes. Further exploration of these effects may provide insight into the physiologic or pathologic role of the placental HERV-E subtypes.

719

A Novel Three-Dimensional In Vitro System of Trophoblast – Endothelium Cell Interaction. Paulomi B Aldo, Graciela Krikun,* Charles J Lockwood,* Gil Mor.* *Obstetrics and Gynecology & Reproductive Science, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: Preeclampsia and IUGR have been linked to improper trophoblast migration that impairs maternal spiral artery transformation minimizing vessel dilation and therefore restricting blood flow to the developing fetus. Endothelial cells are thought to play an essential role in directing trophoblast migration and transformation, although the mechanism by which this occurs is not fully understood. We have developed a novel in vitro system that evaluates endothelial-trophoblast interaction and signaling in a three-dimensional environment.

Methods: An immortalized human endometrial endothelial cell line (HEEC) (Krikun et al., 2004) and first trimester trophoblast cell line HTR-8 were used. Matrigel Basement Membrane Matrix was used for endothelial differentiation into vessel-like structures. Trophoblast migration and transformation was monitored using cell membrane linker dye and OpenLab Image Analysis software. Cytokine and chemokine production was determined using a Beadlyte Multiplex Assay with detection and analysis using the Luminex 100 IS.

Results: Upon differentiation in matrigel, endothelial cells undergo a shift in cytokine production characterized by an increased secretion of chemokines. First trimester trophoblast cells migrate toward the differentiated endothelium, and reach it within 4 to 8 hours. Complete replacement of the endothelium is seen by 48 hours. The transformed tubes maintain structure and remain functional for several weeks allowing for further co-culture studies.

Conclusion: We report the characterization of a novel three-dimensional in vitro system of trophoblast–endothelium cell interaction. We found significant changes in the phenotype (i.e. cytokine expression and response to LPS) of endothelial cells upon differentiation in matrigel as compared to the monolayer culture. These changes may be necessary for the endothelium to direct trophoblast migration. Furthermore, this model represents an ideal co-culture system to evaluate the role of additional cell types, e.g. immune cells, involved in the process of spiral artery transformation.

720

The Inhibitory Effect of Δ^9-Tetrahydrocannabinol on Trophoblast Cell Proliferation and Transcription Is Mediated Mainly Via the CB-2 Receptor. Anthony H Taylor,* Muna S Abbas, Stephen C Bell, Justin C Konje.* *Reproductive Sciences Section, Cancer Studies & Molecular Medicine, University of Leicester, Leicester, Leicestershire, United Kingdom.*

Previously, we have demonstrated that Δ^9-tetrahydrocannabinol (THC), in physiologically relevant concentrations, inhibited the growth and tight transcriptional control of the BeWo trophoblast cell. We sought to find the receptor that mediated this effect and thus treated BeWo cells with up to 30μM THC in the presence and absence of the CB1-, CB2- and VR1-specific antagonists, SR141716A, SR144528 and capsazepine. The results showed that the 40% reduction in cell number after 48 hr treatment with 30 μM THC was only abrogated with SR144528 and not by either SR141716A or capsazepine. Interestingly, capsazepine alone caused a 35% reduction in cell number at 48 hr that was not further enhanced by the presence of THC. These data suggested the presence of a functional CB2 and VR1 receptor, but not a functional CB1 receptor in BeWo cells. RT-PCR with gene specific primers confirmed the presence of transcripts for CB2 and VR1 receptors but not for the CB1 receptor, supporting this conclusion. Although THC caused a 1.7-fold increase in the levels of the transcriptional regulator HDAC3, both VR1 and CB2 antagonists prevented this effect, suggesting that HDAC3 expression and BeWo cell proliferation are causally linked. The inhibitory effect of capsazepine alone on BeWo cell proliferation suggests that BeWo cells make or respond to an endogenous cannabinoid through the VR1 receptor that promotes cell growth/survival. These data imply that the regulation of trophoblast growth and function in response to exo- (and presumably) endo-cannabinoids involves not only the cannabinoid receptors but also the vanilloid receptors.

721

Expression of Homeobox Genes in Placentas with Thrombophilias: Significant Overexpression of HOX A2 Gene. Francesca Castiglione,[1] Federico Mecacci, Benedetta Mangani, Maria A Buccoliero, Francesca Garbini, Cristina Gheri, Duccio Rossi Degl'Innocenti, GianLuigi Taddei, Michael Paidas. (SPON: Charles J Lockwood). *[1]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [2]Department of Gynecology, Perinatology and Reproductive Medicine, University of Florence, School of Medicine, Florence, Italy; [3]Department of Gynecology, Perinatology and Reproductive Medicine, University of Florence, School of Medicine, Florence, Italy; [4]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [5]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [6]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [7]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [8]Department of Human Pathology and Oncology, University of Florence, School of Medicine, Florence, Italy; [9]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background and Objective: Homeobox genes (HOX) are a family of regulatory genes. They play a critical role in the regulation of cellular differentiation. The aim of this study was to investigate the role of HOX genes A2, A4, 9, C6 in human placenta.

Material and Methods: HOX genes mRNA expression was assessed in fresh tissue of human placenta from 12 consecutive placentas of thrombophilic pregnancy (TP) and from 10 human placenta following a normal term birth at the Univ Florence. RNA amplification was assessed by reverse-transcription polymerase chain reaction assay and measured quantitatively. mRNA was successfully extracted in all cases.

Results: Expression of HOX-A2, HOX-A4, HOX-A9 and HOX-C6 genes were detected resp in 66%, 50%, 91%, 100% of the placentas from TP, and resp. in 50%, 20%, 90%, and 90% of the normal placentas. HOX-A2 expression showed significant correlation between placentas from TP and the mean of normal placentas (P=0,05 Kruskal-Wallis test), whereas no correlation was found in expression of HOX-A4, HOX-A9 and HOX-C6 genes between placentas from TP and the mean from the normal placentas.

Conclusion: The presence of HOX genes and the correlation of HOX-A2 gene expression levels between placentas from TP and normal placentas support the hypothesis that this genes may be involved in the regulation of cellular differentiation.

722

Resistin Role on Glucose Transport of Human Placenta. Nicoletta Di Simone, Marco D'Asta, Fiorella Di Nicuolo, Silvia D'Ippolito, Roberta Castellani, Alessandro Caruso. (SPON: Irene Cetin). *Obstetrics and Gynaecology, Catholic University of Sacred Heart, Rome, Italy.*

Objective Resistin is a recently discovered hormone associated with insulin resistance. The increase in serum resistin levels in the third trimester of pregnancy might contribute to the decreased insulin sensitivity in the latter half of pregnancy, which may relate to the development of postprandial hyperglycemia. Glucose transport across the human placenta takes place via GLUT-1 glucose transporters embedded in both microvillous and basal membranes of the syncytial barrier. The aim of this study was to determine resistin role on placental glucose uptake and on GLUT-1 protein regulation, through an induction of MAPK activation.

Materials and Methods BeWo choriocarcinoma cells were cultured in a medium containing resistin (0-100 ng/ml). The immunofluorescence analysis of the BeWo cells was performed by incubating cells with anti-GLUT-1 antibody. Glucose uptake was measured using [^{3}H]-2-deoxy glucose (2-DG). The expression of GLUT-1, total Extracellular signal-Regulated kinase 1-2 (ERK) or phospho-ERK (pERK) were studied by Western Blot analysis and by Real-Time RT-PCR. Quantitative determination of ERK activity was performed by an Enzyme Immunometric Assay (EIA) kit.

Results The immunofluorescence analysis of the BeWo cells revealed a positive GLUT-1 staining in intracellular membrane fractions. Treatment with resistin (10ng/ml) led to a stimulation of 2-DG uptake, while higher concentrations significantly impair basal glucose uptake. Western Blot analysis showed a significant increase of GLUT-1 expression at dose of 10ng/ml. The western blot analysis showed the increase in pERK 1-2 after 20-30 minutes only at 10ng/ml, while total ERK1-2 expression was not affected by the treatment. This data were confirmed by real-time RT-PCR. The EIA assay of ERK activity showed the maximal increased of ERK 1-2 phosphorylation in Bewo cells treated with resistin (10 ng/ml) after 20min of treatment with a decrease at 60min.

Conclusions The present study provided the first evidence that resistin can modulate trophoblast glucose transport. We have demonstrated that resistin causes activation of ERK1-2 pathway in trophoblast cells. Their activation stimulated GLUT-1 synthesis and resulted in increase of placental glucose uptake. High resistin levels (50-100 ng/ml) seem to be able to impair GLUT-1 expression and glucose uptake, reflecting a complex interaction of this adipocytokine with placental functions.

723

Expression and Localization of Phosphorylated mTOR in the Human Placenta; Regulation by the Akt Signaling Pathway. Victoria HJ Roberts, Rose P Webster, Brad A Pitzer, Leslie Myatt.* *Obstetrics & Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

Introduction: The mammalian target of rapamycin (mTOR) is a key component in a signaling pathway that regulates cell growth. In the placenta mTOR is proposed as a growth signaling sensor and some downstream effects have been demonstrated. The active form of mTOR is phosphorylated (pmTOR) and this is thought to be regulated by the serine/threonine kinase Akt. Neither expression of pmTOR nor its regulation have been examined in the placenta. The objectives of this study were to examine pmTOR expression and localization and to elucidate the mechanism by which mTOR is activated in the human placenta.

Methods: Placental tissue was collected immediately following delivery from normotensive (n=8) and severe preeclamptic (PE, n=6) patients. Villous tissue was sectioned, immunostained with p.mTOR primary antibody (1:50) using VectaStain Elite ABC kit and counterstained with hematoxylin (n=3/group). Villous tissue was homogenized in lysis buffer containing protease inhibitors and centrifuged at 20,000xg for 5 min to remove cell debris. mTOR protein was immunoprecipitated from the supernatant using the mTOR specific antibody. An in vitro kinase assay using 1mg recombinant active Akt was performed at 30°C for 30 min. The kinase assay product and total placental protein were separated on SDS-PAGE, probed with the pmTOR primary antibody (1:500) and detected using enhanced chemiluminescence.

Results: Immunohistochemistry demonstrated pmTOR expression throughout the syncytiotrophoblast with no staining observed in the stroma and minimal staining in the endothelial cells. Western analysis of pmTOR showed no significant difference between normal and severe PE although there was a trend towards increased expression in PE. Preliminary data demonstrated phosphorylation of mTOR from placenta by active Akt. Three pmTOR protein bands were detected in the kinase assay samples and these may correspond to the different mTOR complexes that are found in vivo.
Conclusions: mTOR is thought to have an important role in trophoblast cell growth and proliferation. It is regulated by many different factors such as nutrients, hypoxia and oxidative stress however the mechanism of its signal activation is unknown in the placenta. We have shown the potential for phosphorylation of mTOR by Akt in the placenta. It is possible that the relative hypoxia and oxidative stress of PE may regulate mTOR phosphorylation by Akt.

724

Nitration May Regulate the Function of the Placental Growth Signaling Sensor, mTOR. Victoria HJ Roberts, Rose P Webster, Brad A Pitzer, Leslie Myatt.* *Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*
Introduction: The mammalian target of rapamycin (mTOR) has been proposed as a placental growth signaling sensor. The activity of mTOR is regulated by several factors including hypoxia and reactive oxygen species (ROS). In preeclampsia (PE), oxidative stress is increased and generation of ROS such as peroxynitrite can result in nitration, a covalent post-translational modification that can alter protein function. We have previously demonstrated increased placental protein nitration in pregnancies complicated by either PE or diabetes. The objective of this study was to determine whether mTOR is a nitrated protein in the human placenta.
Methods: Placental tissue was collected immediately following delivery from normotensive (n=6) and severe PE (n=6) patients. Villous tissue was homogenized in lysis buffer containing protease inhibitors and centrifuged at 20,000xg for 5 min to remove cell debris. Nitrated proteins were immunoprecipitated (IP) from the supernatant using anti-nitrotyrosine (ANT) antibody (Negative IP controls contained no ANT antibody). Nitrated proteins were separated on SDS-PAGE, probed with a specific mTOR primary antibody (1:500) and detected using enhanced chemiluminescence. The blot was stripped and re-probed with the mTOR antibody pre-incubated with the specific blocking peptide to show band specificity.
Results: Immunoprecipitation demonstrated that mTOR is a nitrated protein in human placental tissue both in normal (n=4 of 6) and severe PE (n=6 of 6) samples. Controls with blocking peptide confirmed that mTOR was specifically detected and negative IP controls gave no bands.
Conclusions: Our results suggest that nitration is a normal post-translational modification of mTOR in the human placenta as nitration was detected in both normal and PE. The mTOR peptide sequence contains 59 tyrosine residues which are all potential targets of nitration and whose modification could effect a conformational change to alter protein structure/function. Of particular interest is Tyr2449 which is located adjacent to Serine 2448, the site of phosphorylation in the active kinase domain which contributes to the catalytic activity of mTOR. It is possible that the extent of nitration is altered with oxidative/nitrative stress and thus the activity/function of mTOR may be compromised which in turn may affect placental and fetal growth.

725

Placental Amino Acid Transport Is Regulated by the mTOR Signaling Pathway. Sara Roos,[1] Theresa L Powell,[1,2] Thomas Jansson.[1,2] (SPON: Leslie Myatt). *[1]Perinatal Center, Dept of Physiology, Institue of Neuroscience and Physiology, Gothenburg University, Gothenburg, Sweden; [2]Dept of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*
Hypothesis: We have proposed that the mammalian target of rapamycin (mTOR) signaling pathway represents a placental nutrient sensor mechanism regulating placental function in response to nutrient availability, and thereby altering fetal growth. Our previous studies have shown that placental mTOR activity is decreased in intrauterine growth restriction (IUGR). We hypothesized that placental nutrient transporters are regulated by mTOR. **Methods:** Cytotrophoblast cells were isolated and cultured. Between 66 and 90 hours of culture, cells were incubated with 100 nM rapamycin, a specific inhibitor of mTOR, or vehicle. Subsequently, amino acid transporter activity was measured by determining the mediated uptake of [^{14}C]MeAIB by system A and [^{3}H]leucine by system L. **Results:** Rapamycin decreased system L activity by 28% ($n = 8$, $P < 0.05$, Wilcoxon Test) and system A activity by 17% ($n = 8$, $P < 0.05$, Wilcoxon Test). **Conclusions:** It has previously been shown that IUGR is characterized by a decrease in the activity of placental amino acid transporters, whereas fetal overgrowth is associated with an increase in placental system A and system L amino acid transporter activity. The finding that inhibition of placental mTOR down-regulates system L and system A activity identifies a novel regulatory mechanism for these transporters and is compatible with the suggestion that mTOR functions as a placental nutrient sensing mechanism.

726

Acute Hypoxia Decreases System A Amino Acid Transporter Activity in Placental Villous Explants. Frauke von Versen-Hoynck,[1,2,4] Augustine Rajakumar,[*1,2] James M Roberts,[*1,2,3] Nina Markovic,[3] Robert W Powers.[*1,2] *[1]Magee-Womens Research Institute; [2]Department of Obstetrics and Gynecology and Reproductive Sciences; [3]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [4]Department of Obstetrics and Gynecology, RWTH Aachen University, Aachen, Germany.*
Human fetal development is dependent upon an adequate supply of amino acids from the maternal circulation. Amino acids are transported across the placenta by several specific transporters that result in two-fold higher amino acid concentrations in the fetal circulation. Fetal growth restriction is associated with lower fetal amino acid concentrations and lower placental amino acid transport. One mechanism by which placental amino acid transport may be adversely affected is villous hypoxia as a result of placental underperfusion.
Objective: To test whether acute hypoxic conditions influence system A amino acid transporter activity in placental villous explants.
Methods: The uptake of C^{14}-methyl-aminoisobuteric acid (MeAIB) (n=3) was determined after incubation of a triplicate set of placental villous explants isolated from placentas of primiparous women with uncomplicated pregnancies for 10, 30 and 120 minutes under hypoxic (2% oxygen) and standard (20% oxygen) conditions. Data are presented as mean± SEM. Statistical analyzes were performed with a paired t-test, probability values were considered significant at $p<0.05$.
Results: Hypoxia significantly decreased system A amino acid transport activity in placental villous fragments by 60%, 72% and 90% after 10, 30 and 120 minutes, respectively (p=0.029, 0.005, 0.0016) compared to standard conditions. The decrease in system A activity was significant within 10 minutes of hypoxia exposure (7.76 ± 0.46 vs. 19.64 ± 0.44 pmol/mg/40min, p=0.029).
Conclusions: Primary villous explants can be used as a model to investigate the effect of lower oxygen concentrations on amino acid transport. Our preliminary data suggest that hypoxia has a remarkably acute effect on placental amino acid transport. Several mechanisms may account for this robust acute effect including changes in transcription, translation or post-translational changes of System A transporter proteins.
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727

Adenosine Reduces System A Amino Acid Transporter Activity in Placental Villous Explants. Frauke von Versen-Hoynck,[1,2,4] Augustine Rajakumar,[*1,2] James M Roberts,[*1,2,3] Gail Harger,[3] Robert W Powers.[*1,2] *[1]Magee-Womens Research Institute; [2]Department of Obstetrics and Gynecology and Reproductive Sciences; [3]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [4]Department of Obstetrics and Gynecology, RWTH Aachen University, Aachen, Germany.*
Background: Adenosine, a metabolite of the adenine nucleotide is produced in response to ischemia and hypoxia in the placenta. Adenosine has been implicated in regulatory processes including regulation of local blood flow, metabolic rate and renal sodium transport by Na+/K+ ATPases. Maternal plasma adenosine concentrations are higher in uncomplicated pregnancies and preeclampsia (PE), with a significantly greater concentration in PE. The activity of the sodium- dependent system A amino acid transporter in the placenta is lower in pregnancies that are complicated by intrauterine-growth restriction but not different in PE compared to uncomplicated pregnancies.
Objective: The focus of this study was to investigate whether adenosine reduces system A amino acid transporter activity in placental villous explants.
Methods: The uptake of C^{14}-MeAIB was investigated after incubation of placental villous explants isolated from placentas of women with uncomplicated pregnancies for 24 hrs with adenosine (1-100µM, n=5) with or without

allopurinol (1mM, n=2). Data are presented as mean±SEM and as percentage of the control. Statistical analysis was performed with student's t-test, values were considered significant at $p<0.05$.
Results: System A activity was 32% and 28% lower after incubation with adenosine (50 and 100 μM, respectively) compared with controls (0.68±0.12 and 0.72±0.1 vs.1.01±0.08 pmol/mg protein/min, p=0.052, 0.056). Allopurinol, a xanthine oxidase inhibitor, did not reverse the adenosine effect on amino acid transporter activity (0.58±0.004 and 0.68±0.12 vs. 1.01±0.08 pmol/mg/min).
Conclusions: Adenosine reduces system A amino acid uptake in villous explants. This effect seems to be independent of xanthine oxidase since allopurinol did not prevent the reduction in transporter activity. The reduction in transporter activity by adenosine might be a consequence of a reduction in Na+/K+ ATPase activity leading to an imbalance of the sodium gradient. However, this results needs to be investigated further.
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728

Leptin Does Not Alter Placental System A Amino Acid Transporter Activity but Activates Its Receptor. Frauke von Versen-Hoynck,[1,2,4] Augustine Rajakumar,[*1,2] James M Roberts,[*1,2,3] Nina Markovic,[3] Robert W Powers.[*1,2]
[1]Magee-Womens Research Institute; [2]Department of Obstetrics and Gynecology and Reproductive Sciences; [3]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [4]Department of Obstetrics and Gynecology, RWTH Aachen University, Aachen, Germany.
Maternal leptin is elevated during pregnancy and preeclampsia, and is postulated to be an important mediator of reproductive function and metabolic regulation. Leptin has previously been shown to increase placental System A amino acid transport activity. Amino acids serve as one of the main nutrient sources for fetal growth, accounting for 20 to 40% of fetal energy requirements.
Objective: To further investigate the influence of leptin on placental amino acid transporter activity.
Methods: The uptake of C^{14}-MeAIB was investigated after incubation of primary villous fragments isolated from placentas of primiparous women with uncomplicated pregnancies for 2 hours in leptin (1ug/ml, n=22) and insulin (300ng/ml, n=5). All incubations were done in triplicate. Activation of the leptin receptor in villous fragments was determined by Western analysis of total and phosphorylated STAT3 (STAT3 and pSTAT3 respectively) (n=9). Transporter activity data are presented as mean±SEM and Western data as percentage of controls. Statistical analyzes was done by Wilcoxon/Kruskal Wallis test, probability values were considered significant at $p<0.05$.
Results: There was no effect of leptin on the activity of System A (1.89±0.24 pmol C^{14} MeAIB/min/mg) amino acid transporter activity in primary villous fragments compared to untreated control fragments (1.91±0.22 pmol C^{14} MeAIB/40min/mg, p=0.86). However, STAT3 phosphorylation was increased by 32% after leptin incubation. Furthermore, in contrast to leptin, incubation with insulin increased System A activity by 34%.
Conclusions: Contrary to previous work, acute leptin incubation does not appear to influence placental System A amino acid transport activity. In contrast, leptin incubation does increase pSTAT3 in villous fragments, and incubation with insulin does increase System A activity.
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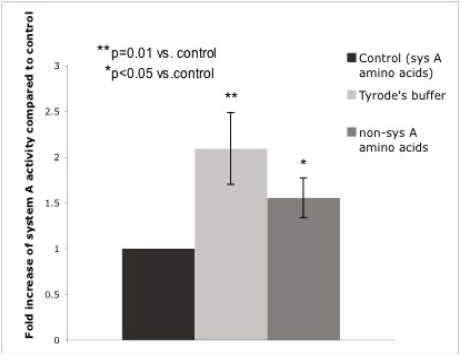
729

Enhanced Placental System A Amino Acid Transporter Activity with Amino Acid Limitation Is Substrate-Specific. Meredith L Snook Parrott,[1,2] Frauke M von Versen-Hoynck,[1,2] Gail Harger,[3] Nina Markovic,[3] James M Roberts.[*1,2,3] *[1]Magee-Womens Research Institute; [2]Obstetrics and Gynecology and Reproductive Sciences; [3]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA.*
Objective: Conditions associated with reduced nutrient availability to the placenta, such as inadequate nutrient intake or preeclampsia, may nonetheless result in normally grown infants. The precise mechanisms for this phenomenon are unknown. We characterized the effect of reduced amino acid availability on amino acid uptake via system A in placental villous fragments. We tested the hypotheses that reduced amino acid availability would increase placental uptake of amino acids to compensate for this deficiency and that the effect would be specific for substrates of the system A transporter.
Methods: Villous explants from ten placentas of uncomplicated, term, singleton pregnancies were incubated for 2 hours in either: (1) amino acid deficient media (AA-), (2) amino acid sufficient media (AA2) containing only amino acids transported by system A (1.0 mM gln, 0.1 mM gly, and 0.1 mM ser), or (3) amino acid sufficient media (AA2-) containing only amino acids not specifically transported by system A (1.0 mM his, 1.0 mM lys, and 1.0 mM arg). System A activity was measured by Na+-dependent uptake of methylaminoisobutyric acid (MeAIB). Data are expressed as fold increase compared to the control (AA2) media (mean + SEM) and were analyzed by Wilcoxon signed-rank tests.
Results: AA- treated fragments had significantly greater system A activity than AA2 treated fragments (2.10 + 0.39, p= 0.01). Similarly, AA2- treated explants had greater amino acid uptake than explants treated with AA2 (1.56 + 0.22, $p<0.05$). There was no difference in system A activity between AA- and AA2- (p=0.14) treatment. (Figure)
Conclusions: The system A transporter of normal placentas upregulates amino acid transport in response to reduced system A substrate. This may compensate for reduced amino acid availability to ensure proper fetal growth.
Funded in part by NIH P01 HD30367. MSP is a medical student in the Clinical Scientist Training Program and is sponsored by a Dean's Fellowship.



730

Endothelial Lipase Is the Predominant Triglyceride-Lipase Family Member in the Human Placenta and Dysregulated in Intrauterine Growth-Restricted Pregnancies but Not in Gestational Diabetes. Christian Wadsack,[1] Martin Gauster,[1] Irene Cetin,[*2] Uwe Lang,[*1] Ursula Hiden,[1] Astrid Blaschitz,[3] Gernot Desoye.[*1] *[1]Dept Obstet & Gynecol, Medical University of Graz, Graz, Austria; [2]Institute Obstet & Gynecol, Foundation IRCCS PoMaRe, University of Milano, Milano, Italy; [3]Institute Cell Biol, Histol & Embryol, Medical University of Graz, Graz, Austria.*
Objective:
Fetal supply of maternally derived fatty acids requires the hydrolysis of lipoprotein-borne triglycerides and phospholipids at the placental surface. This study tested the hypothesis that members of the triglyceride lipase gene (TLG) family are expressed in human placenta at the materno-placental (syncytiotrophoblast) and feto-placental (endothelial cells) interface and that their expression is altered in pregnancy pathologies.
Methods:
Expression of TLG family members in first trimester and term human placenta, trophoblast and endothelial cells was analyzed by microarrays, RT-PCR, Western blotting and immunohistochemistry. Their expression was compared between normal pregnancies and those complicated with gestational diabetes and intrauterine growth-restriction (IUGR).
Results:
Only endothelial lipase (EL) and lipoprotein lipase (LPL) were expressed in placenta. EL expression was 7-fold higher ($p<0.001$) than LPL and remained similar at both gestational periods. LPL mRNA levels were 2.6-fold higher ($p<0.005$) at term as compared to the first trimester. In first trimester both lipases were expressed in villous trophoblasts (FT). Only EL was detected in extravillous trophoblasts. At term EL was detected in the trophoblasts (TT) and in endothelial cells (EC), while LPL was only found in smooth muscle cells. After heparin release of EL and LPL at the cell surface of FTs, TTs and ECs (functional lipases) only EL was detected on all cell types, whereas LPL was only detected in control cells overexpressing LPL. TNF-alpha increased EL mRNA *in vitro* whereas oxygen, insulin and glucose did not change EL and LPL expression. Compared with normal placentas, EL mRNA was decreased (30%; $p<0.02$), while LPL mRNA expression was increased (2.4-fold; $p<0.015$)

SATURDAY

in IUGR. EL and LPL expression was unaltered in diabetic placentas. Placental EL, but not LPL, expression levels were negatively correlated with maternal pre-gestational BMI.

Conclusion:

EL is the predominant TLG family member in the human placenta present at both interfaces. EL and LPL are dysregulated in IUGR but not in diabetes.

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731

Different Pattern of Expression of Wolframin in Normal and Diabetic Human Placenta. M De Falco,[1] N Colacurci,[2] A Mastrogiacomo,[2] E Federico,[2] G Acone,[2] A Gatto,[2] A Colonna,[2] G Coppola,[3] A De Luca,[3] L Cobellis.[2] (SPON: Felice Petraglia). *[1]Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, Federico II University, Naples, Italy; [2]Dept. of Obstetrics and Gynecology, II University of Naples, Naples, Italy; [3]Dept. of Medicine and Public Health, Section of Clinical Anatomy, II University of Naples, Naples, Italy.*

Hypothesis. The WFS1 gene,encoding a transmembrane endoglycosidase H-sensitive glycoprotein of the endoplasmic reticulum called wolframin,is mutated Wolfram syndrome,defined by the association of diabete mellitus,optic atrophy,and further organ abnormalities.It has been demonstrated that Wfs1 is normally up-regulated during insulin secretion,whereas disruption of the Wfs1 gene in mice,causes progressive beta-cell loss within the pancreas and impaired stimulus-secretion coupling in insulin secretion.The physiological function of this protein remains unknown.Human placenta is unique in many aspects of its growth and differentiation,including the requirement to invade another organ,the uterus,for survival.Due to the correlation between Wfs1 mutation and diabetes,we investigated immunohistochemical expression of wolframin in human placenta throughout pregnancy in normal and diabetes. **Methods**Placenta samples at I trimester of pregnancy were from patients with spontaneus miscarriage(n.15).A total of 60 samples were from patients undergoing voluntary pregnancy termination:n.15 from normal patients and n.15 from diabetic patients.The specimens were immediately fixed in formalin for ihc.**Results.**In normal placenta, wolframin was localized, at strong level of expression, in the cytoplasm of CT and endothelial cells during the I trimester. In the III trimester,wolframin was present at moderate level of expression, in the cytoplasm of CTcells and in endothelial cells inside placental villi.In diabetes,the wolframin expression was strongly reduced in the III trimester. Moreover,this protein was localized almost exclusively in the stroma of placental villi.**Conclusion.**The pattern of expression of wolframin in normal placenta through gestation, suggests that this protein may be required to sustain normal rates of CT cell proliferation during the I trimester of gestation.The decrease of wolframin expression in diabetic placenta may hypothesize that this protein is directly regulated by insulin concentration also in the placenta, suggesting that this protein physiologically maintain the glucose homeostasis in this organ.

732

Abstract Withdrawn.

733

Glucocorticoid Metabolism Enzyme Expression Changes in Rat Placenta during Maternal Food Restriction. Andrea Jelks, Guang Han, Michael G Ross,* Mina Desai. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

BACKGROUND: Maternal food restriction (MFR) during pregnancy results in low birth weight newborns which develop metabolic syndrome as adults. MFR increases maternal plasma glucocorticoids (GC) and thus programming effects may result from increased fetal GC exposure. In the rat, placental 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive 11-dehydrocorticosterone into active corticosterone, thus potentiating transfer of maternal steroids to the fetus; 11β-HSD2 does the opposite and acts as the physiological feto-placental "barrier" to maternal glucocorticoid. The placental labyrinth zone is the major site of maternal-fetal exchange whereas the basal zone is the primary site of placental hormone synthesis. We determined the impact of maternal food restriction (MFR) on placental expression of 11β-HSD isoforms in the two placental zones.

METHODS: Control dams (n=12) received ad libitum food whereas study dams were food restricted (MFR, n=12) from pregnancy day 10. At E16 and E20, placentas were separated into basal and labyrinth zones, and mRNA expression of glucocorticoid receptor, and 11β-HSD1 and 11β-HSD2 enzymes were determined using real time RT-PCR. Data was normalized to β-actin and values are shown as mean fold difference ±SE.

RESULTS: At E16, expression of 11β-HSD1 was significantly reduced in the basal zone of MFR placentas (0.47±0.3 fold, $p<0.01$), though no significant change was seen in the labyrinth zone. At E20, expression of 11β-HSD2 was reduced in basal zone but not in labyrinth zone in MFR placentas. There was no effect of MFR on placental expression of 11β-HSD2 at E16, or 11β-HSD1 at E20. The glucocorticoid receptor was similarly expressed in the basal and labyrinth zone at both E16 and E20 in MFR and Control placentas.

CONCLUSION: Decreased expression of placental 11β-HSD1 in the E16 basal zone reduces the capacity of conversion of inactive to active GC. This may serve as an important barrier, reducing transplacental transfer of excess steroids to the fetus during MFR. Conversely, decreased 11BHSD-2 expression in the basal zone at E20 may increase exposure of the fetus to maternal GC near term. These results suggest that GC-induced programming effects of MFR may occur in late gestation (i.e., following E16).

734

Maternal Dietary Protein Restriction and Gene Expression Changes in the Murine Placenta. Ciprian P Gheorghe,[1] Subburaman Mohan,[2] Lawrence D Longo.*[1] *[1]Center for Perinatal Biology, Departments of Physiology and Obstetrics and Gynecology, Loma Linda University, Loma Linda, CA, USA; [2]Musculoskeletal Disease Center, Jerry L. Pettis VA Medical Center, Loma Linda, CA, USA.*

OBJECTIVE: Maternal protein restriction has been shown to have deleterious effects on placental development, and has long-term consequences for the progeny. To test the hypothesis that maternal protein restriction triggers gene expression changes, we measured these in the mouse placenta.

METHODS: We exposed pregnant FVB/NJ mice from embryonic day 10.5 (E10.5) to E17.5, to an isocaloric diet containing 50% less protein than normal chow (10% vs. 20% protein content). Following RNA extraction, we used the Affymetrix Mouse 430A_2.0 array to measure gene expression changes. We observed 244 probe sets, corresponding to 235 genes, regulated by protein restriction ($p < 0.001$). Ninety-one genes were up-regulated and 153 were down-regulated. We functionally annotated the regulated genes, and examined over-represented functional categories.

RESULTS: Among the up- and down-regulated genes, we observed several over-represented functional categories. Up-regulated genes included those involved in apoptosis, negative regulators of cell growth, negative regulators of cell metabolism and genes related to epigenetic control. Down-regulated genes included those involved in nucleotide metabolism.

CONCLUSIONS: Microarray analysis has allowed us to describe the genetic response to maternal protein deprivation in the mouse placenta. Apoptosis-related genes, negative regulators of cell growth and metabolism, and genes involved in epigenesis were up-regulated. This suggests a mechanism through which maternal protein deprivation contributes to growth restriction and long-term epigenetic changes in stressed tissues and organs. (Supported by USPHS HD-3807).

735

Maternal Baboon 30% Global Nutrient Restriction (MNR) from 30 to 165 Days of Gestation (dG) Decreases Trophoblast Leptin and Leptin Long Form Receptor (Ob-R) Protein Expression in Placenta. Cun Li, Natalia E Schlabritz-Loutsevitch, Mark J Nijland, Thomas J McDonald,* Peter W Nathanielsz.* *Center for Pregnancy and Newborn Research, Dept. Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, TX, USA.*

Introduction: In humans, leptin is: 1) produced by placental trophoblast, 2) secreted into both maternal and fetal circulation, 3) increased in diabetic pregnancy and pre-eclampsia and 4) reduced in maternal and fetal plasma in intrauterine growth restriction (IUGR; Placenta 23:103). While umbilical blood leptin is strongly correlated with fetal fat mass [AJOG 187:798], no data exist for placental leptin changes in MNR throughout gestation. The present study examined MNR effects on baboon placental leptin and Ob-R protein expression at 165dG. We previously reported that MNR delays leptin maturation in placental villi at 90 dG [2005 DOHAD P. 1-084]. **Methods:** Pregnant baboons were fed ad libitum (CTR; n = 7) or fed 70% of CTR diet (NR; n = 5) from 30 - 165 dG with C-section and tissue collection at 165 dG. Standard immunocytochemistry was performed using leptin and long form Ob-R antibodies (1:50 final dilution). Photomicrographs taken at 20X were analyzed for fraction (area immunostained ÷ area of the field x 100%) by image analysis. Statistical analysis was performed with Rank sum test for single comparisons with α level set at 0.05. All data are expressed as mean ± SEM.

Results: Immunoreactivity was found in both syncytiotrophoblast (ST) apical membrane (AM) and in ST cytoplasm (CP) (Table 1). **Conclusion:** While the functions of placental leptin remain to be precisely determined, MNR results in significant decreases in leptin and its receptor at 165 dG suggesting a reduction in function by decreased nutrient availability at this gestational age.

Table 1. Leptin and leptin receptor (OB-R) protein expression in apical membrane (AM) and cytoplasm (CP) of syncititotrophoblast at 165 days of gestation in control (CTR) vs. nutrient restricted (MNR) baboons.

	Leptin		OB-R	
	AM	CP	AM	CP
CTR	85.4±3.3	65.7±7.6	96.9±0.4	64.3±4.0
MNR	34.9±11.7*	21.2±8.3*	73.3±2.4*	28.3±8.9*

AM - apical membrane; CP - cytoplasm * Different from control, p< 0.05

736

Use of BeWo Cells as an In-Vitro Placental Model To Study Glucose Uptake in Response to Myostatin. Nisha Antony,[1,2] Murray D Mitchell,[*1,2] John J Bass.[1,2] *[1]Liggins Institute, University of Auckland, Auckland, New Zealand; [2]National Research Center for Growth and Development, C/-Liggins Institute, Auckland, New Zealand.*

BACKGROUND: Myostatin is a member of the TGF-ß superfamily and has diverse biological functions. Metabolism and regulation of glucose uptake are key actions of myostatin in skeletal muscle.

OBJECTIVE: To examine the effects of myostatin treatments on glucose uptake by a placental cell line, (BeWo), in which myostatin has been identified.

METHODS: BeWo cells were seeded at densities of $0.5x10^5$ cells/well in 24 well plates in DMEM:HAMsF_{12} [1:1], supplemented with 10% FCS, 1% Penicillin/ Streptomycin and 1% Glutamax. Following 24hrs, 48hrs, 72hrs and 96hrs of incubation, cells were treated with [1µCi/ml 2-Deoxy-d-[1-^{14}C]] glucose in Tyrode solution and 0.2nM, 0.4nM, 4nM and 40nM of recombinant human myostatin and/or follistatin for 20min, at 37^{O}C, in a humidified incubator maintained at 5% CO_2. After incubation, cells were lysed and radioactivity was measured in a liquid scintillation analyser and normalised with protein concentration per well.

RESULTS: Myostatin inhibited glucose uptake in a concentration dependent manner following 24hrs of seeding (P<0.01, n=4 for each treatment). Follistatin is a functional inhibitor of myostatin. Following treatment with 40nM myostatin and various concentrations of follistatin, the decrease in glucose uptake that occurs with myostatin treatment alone was reversed (0.4nM follistatin treatment, P<0.05, n=4 for each treatment). Follistatin treatment alone increased glucose uptake (0.4nM and 4nM, P<0.001, 40nM P<0.05; n=4 for each treatment). Interestingly irrespective of treatment, glucose uptake declined as cells proliferated and greater cell densities were achieved (P<0.001). Data were compared only between treatments and controls at each individual time point.

CONCLUSIONS: These findings implicate a possible metabolic role of myostatin and follistatin on nutrient uptake in the feto-placental-maternal units. Altered myostatin actions in the placenta may have implications in pregnancies complicated by placental insufficiencies, in particular intra- uterine growth restriction (IUGR), preterm birth and pre-eclampsia and offers a potential therapeutic target.

737

Cell Proliferation or Cell Death — The Role of Telomeres in Pre-Eclampsia. Yali Xiong,[1] Dan Liebermann,[2] Eli J Holtzman,[3] Barbara Hoffman,[2] Enrique Hernandez,[1] Ossie Geifman-Holtzman.[1] (SPON: Ira M Bernstein). *[1]Obstetrics & Gynecology, Temple University, Philadelphia, PA, USA; [2]Biochemistry, Fels Institute, Temple University, Philadelphia, PA, USA; [3]Nephrology and Hypertenstion Institute, Sheba Medical Center, Tel-Aviv University, Ramat-Gan, Israel.*

OBJECTIVE: To examine the biologic behaviour of telomeres via the activity of telomerase in pregnant patients with preeclampsia

STUDY DESIGN: Placenta tissue samples from 20 patients with pre-eclamspia in the third trimester of pregnancy (study group) and 20 healthy pregnancies (control group) were freshly collected and preserved in RNAlater reagent. Total RNA was isolated from placenta tissue and reversely transcribed to c-DNA. Specific probe for real-time PCR was designed according to the sequence of the full length c-DNA of telomerase reverse transcriptase (TERT). The mRNA level of TERT was detected with a probe-specific real-time quantitative PCR assay using the RT products as templates. ß-actin was applied as a reference gene when Cp values were obtained during the real-time polymerase chain reaction to determine the relative expressional levels of TERT.

RESULTS: Telomerase reverse transcriptase (TERT) expression was present in the placentas of both pre-eclamptic and normal pregnancies. The mRNA level of TERT was 70% higher in the placentas of pre-eclamptic patients than in the normal placentas (N=20, p<0.05). This increased expression of TERT reflects on increased telomerase activity.

CONCLUSION: Increased mRNA level of TERT indicates a higher activity of telomerase in pre-eclampsia than in normal pregnancies which stands for increased cell activity and proliferation. This finding may suggest that cells obtained from placenta with pre-eclampsia are more likely to demonstrate dysfunctional proliferation rather than programmed cell death, apoptosis.

738

Recruitment of Macrophages and Dendritic Cells into the Decidua and Their Role in the Pathogenesis of Preeclampsia. S Joseph Huang,[1] Chie-Pein Chen,[2] Frederick Schatz,[*1] Vikki M Abrahams,[1] Charles J Lockwood.[*1] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University, Hew Haven, CT, USA; [2]Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan.*

Objective: Cytotrophoblast (CT) invade decidua embedded with low levels of macrophages (Macs) and dendritic cells (DCs). Both Macs and DCs mediate innate immunity, adaptive immunity and promote immune tolerance. An imbalance between defense against pathogens and tolerance of the semi-allogeneic embryo in the decidua promotes preeclampsia (PE). PE is associated with a restricted CT invasion and impaired spiral artery remodeling. We hypothesize that pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) dysregulate trafficking and activation of decidual Macs and DCs by targeting decidual cells to synthesize and secrete relevant chemokines and colony stimulating factors (CSFs). The resulting influx of Macs and DCs impairs CT invasion.

Methods: Immunostaining was used to localize Macs, DCs, chemokines and CSFs in normal versus PE decidua. Leukocyte-free first trimester decidual cells were treated with E_2 and MPA +/- 1 ng/ml TNF-α or IL-1β for 6 hrs. Total RNA was extracted for microarray analysis and real time RT-PCR. Western blotting and ELISA measured protein levels in conditioned media.

Results: These results extend our previous observation for Macs by revealing that compared to normal decidua, the preeclamptic decidua basalis also contains a dense infiltration of DCs (n=8, p < 0.05). Expression of an array of Mac- and DC-recruiting chemokines as well as potent Mac and DC activators, M-CSF and GM-CSF, were enhanced in the preeclamptic decidua (n=8, p < 0.05). In cultured first trimester decidual cells, TNF-α and IL-1β markedly up-regulated the expression of CCL2, CCL4, CCL5, CCL7, CCL20, CXCL8, M-CSF and GM-CSF (5- to 3800-fold, n=3, p < 0.05).

Conclusions: These results support the pivotal roles played by TNF-α- and IL-1β- induced chemokines and CSFs in recruiting and activating Macs and DCs at the implantation site of patients destined to develop PE.

739

The Characteristic Changes in the Endothelial Function by Administration of L-Arginine Plus Folic Acid in Preeclampsia. Yoshikatsu Suzuki,[1] Tamao Yamamoto,[1] Nobuhiro Suzumori,[1] Mayumi Sugiura,[1] Hidetaka Izumi.[*2] *[1]Obstetric and Gynecology, Nagoya City University Medical Sciences, Nagoya, Japan; [2]Obstetric and Gynecology, Izumi Women Hospital, Fukuoka, Japan.*

It was found that the reduced action of nitric oxide (NO) was seen in nitroglycerin treated rabbit, since the increased production of superoxide might change the function of endothelial NO synthase. The administration of L-arginine plus folic acid might improve the reduced action of NO due to an increase in the concentration of L-arginine in the endothelium and inhibition of oxidization of tetrahydrobiopterin by folic acid. In this study, we investigated the characteristic changes in endothelial function by the administration of L-arginine and folic acid in preeclamptic women compared with normotensive pregnant women. Ten non-pregnant women, ten normotensive healthy pregnant women, ten normotensive pregnant women at high risk group of preeclampsia (previous preeclampsia and complicated by hypertension or diabetes) were participated. Preeclampsia was diagnosed according to pregnancy-induced hypertension criteria as Japan Society of Obstetrics and Gynecology. In four groups, L-arginine (3g/day) plus folic acid (10 mg/day) was administrated and flow mediated vasodilatation (FMD) of brachial arteries by hyperemia was observed on 48 hour after the administration. In non-pregnant, high risk pregnant and preeclamptic women, FMD was lower than that of normotensive pregnant women. By the administration, in non-pregnant women and high risk pregnant women, the FMD was increased and restored similar level of normal pregnant women, while it was not changed in preeclamptic women. It was concluded that the supplementation might prevent preeclampsia due to restoring or enhancing reduced endothelial function.

740

Elevated Maternal Serum Levels of MMP2 and Increased Ratio of MMP2/TIMP2 in Women with Preeclampsia Compared to Those in Women with Normal Pregnancies. Kevin Hooker, David F Lewis, Yang Gu, Lisa Philibert, Yanping Zhang, Yuping Wang.* *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: Matrix metalloproteinases (MMPs) exert diverse effects on vascular tissue remodeling and inflammatory responses during normal pregnancy. Altered MMP(s) and their native inhibitor TIMP(s) levels are believed to be involved in endothelial activation/dysfunction and vascular pathophysiology during preeclampsia (PE). The purpose of the study was to determine whether maternal MMP2 and TIMP2 levels and their ratios were different between normal pregnant women and women with PE.

Study Design: Venous blood was obtained from 80 women at the time of admission to the Labor and Delivery Unit at LSUHSC-Shreveport (PE: n=41; normal pregnancy: n=39). The criteria for normal pregnancy and PE are followed by ACOG guidelines. Serum concentrations for MMP2 and TIMP2 were measured by enzyme-linked immunosobent assay (ELISA). All samples were assayed in duplicate. Data are presented as mean ± SE and analyzed by nonparametric Mann Whitney test. Power analysis was performed with statistical software (Power and Precision). A p level less than 0.05 is set for statistically different.

Results: Maternal serum MMP2 levels were significantly higher in women with PE than in women with normal pregnancies, 479.09 ± 23.78 vs. 351.01 ± 13.78 ng/ml, $p < 0.01$, with Power = 0.995, Alpha = 0.05, Tail = 2, respectively. There was no difference for serum TIMP2 levels between PE and normal pregnancies, 244.93 ± 15.24 vs. 234.52 ± 25.39 ng/ml, $p > 0.1$. The ratio of MMP2/TIMP2 was higher in PE than in normal pregnancies, 2.31 ± 0.19 vs. 1.91 ± 0.17, $p < 0.05$, respectively.

Conclusions: Elevated maternal serum levels of MMP2 may be a component of endothelial activation/dysfunction during preeclampsia since activated endothelial cells release MMP2. TIMP2 is a native receptor for MMP2. The increased ratio of MMP2/TIMP2 may play a role in altered vascular remodeling and reactivity in the maternal syndrome of PE during pregnancy.

741

Placental Hypoxia-Inducible Factor 1α in Pre-Eclampsia and Cytotrophoblast Regulation. Xilian Bai, Alexander EP Heazell, Jenny E Myers, Philip N Baker,* John D Aplin, Ian P Crocker. *Division of Human Development, University of Manchester, United Kingdom.*

Objectives: Altered trophoblast cell turnover in pre-eclampsia (PE) is associated with attenuated placental oxygen (O_2). Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor which functions as an adaptor to O_2 deprivation. The role of HIF-1α in modulating proliferation, differentiation and apoptosis in the human placenta is unclear. We have evaluated placental HIF-1α and its regulator prolyl-hydroxylase 2 (PHD2) in pre-eclampsia and defined their roles in determining villous cytotrophoblast (CT) phenotype.

Methods: Placentae were obtained from women undergoing elective Caesarean section; 8 PE and 10 from uncomplicated normal pregnancies (NP). Fresh tissue was investigated and villous explants cultured for 4 days in 1%, 6% or 20%O_2, to mimic placental hypoxia, normoxia and hyperoxia. Additional explants were cultured for 3 days at 6%O_2 and treated for 24hrs with $CoCl_2$ at 0, 50, 100μM, to initiate HIF-1α stabilization. On day 4, CT apoptosis and differentiation were assessed by TUNEL and hCG production, and tissue viability by liberated lactate dehydrogenase (LDH). Protein expression and localisation of HIF-1α and PHD2 were monitored by Western blotting and immunohistochemistry.

Results: HIF-1α was abundantly expressed in normal villous tissue, particularly localised to CT and placental capillaries. PHD2 was strongly expressed in CT, syncytiotrophoblast (ST) and weakly in the stoma. No differences in HIF-1α and PHD2 expression/localisation were evident in normal pregnancies compared to PE. In 1%O_2, TUNEL postivity was increased in ST and CT compared to 6 and 20%O_2 ($p<0.05$, Friedman test); LDH was increased ($p<0.05$) and hCG reduced ($p<0.05$). HIF-1α remained unaffected by O_2 exposure. Tissue responses to O_2 were consistent between NP and PE. HIF-1α and PHD2 were unchanged, tissue viability was unaffected, but hCG production was significantly elevated at all O_2 conditions. HIF-1α was increased by $CoCl_2$, but no changes in either apoptosis or differentiation of CT were apparent. Conclusion: HIF-1α is abundant in the normal placental villus, particularly CT. *In vitro,* O_2-dependent changes in CT phenotype were independent of HIF-1α. The similarities in expression and responses between normal and PE pregnancies would refute the importance of placental HIF-1α in this condition. We would speculate that high levels of intrinsic HIF-1α protect the placenta against O_2 deprivation *in vivo*.

742

Tissue Factor and Its Natural Inhibitor in Preeclampsia and SGA. Offer Erez,[1] Debra Hoppensteadt,[2] Nandor Gabor Than,[1] Jawed Fareed,[2] Shali Mazaki-Tovi,[3] Jimmy Espinoza,[1,3] Tinnakorn Chaiworapongsa,[3] Bo Hyun Yoon,[4] Sonia Hassan,[1,3] Francesca Gotsch,[1] Juan Pedro Kusanovic,[1] Roberto Romero.*[1,5] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Pathology, Loyola Medical Center, Maywood, IL, USA; [3]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics and Gynecology, Seoul National University, Seoul, Korea; [5]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA.*

OBJECTIVE: Tissue factor (TF), the major activator of the extrinsic pathway of coagulation, is abundant in the placenta and decidua. The aim of this study was to determine the maternal plasma concentrations of TF and its primary inhibitor, tissue factor pathway inhibitor (TFPI) in women who delivered small for gestational age (SGA) neonates and in preeclampsia.

STUDY DESIGN: A cross sectional study included the following groups: 1) normal pregnancies (n=71); 2) women who delivered SGA neonates (n=58) and 3) women with preeclampsia (n=130). Plasma concentrations of TF and TFPI were measured by a sensitive immunoassay. Non-parametric statistics were used for analysis.

RESULTS: 1) Women with preeclampsia had a significantly higher median plasma concentration of TF than patients with normal pregnancies (median: 1187pg/ml; range: 567-11675 vs. median: 345.7 pg/ml; range: 21.7-2660.2; $p<0.0001$, respectively); 2) Similarly, TFPI concentrations were higher in preeclampsia than in normal pregnancies (median: 87.55ng/ml; range 25.4-165.1 vs. median: 66.7ng/ml; range: 37.4-86.5; $p<0.0001$, respectively); 3) Surprisingly, mothers with SGA neonates had a lower median maternal plasma concentration of TF (median: 102.4pg/ml; range: 25.6-1225.3) than women with normal pregnancies ($p<0.0001$).

CONCLUSION: 1) Maternal plasma concentrations of TF in preeclampsia but not SGA were higher than in normal pregnancies; 2) While the role of immunoreactive plasma TF concentration in coagulation remains controversial, our observations suggest that changes are present in the context of complications of pregnancy.

743

The Expression of Gadd45 Genes and P38 Activation in Preecalmpsia. Yali Xiong,[1] Dan Liebermann,[2] Barbara Hoffman,[2] Eli J Holtzman,[3] Enrique Hernandez,[1] Ossie Geifman-Holtzman.[1] (SPON: Ira M Bernstein). *[1]Obstetrics/Gynecology, Temple University, Philadelphia, PA, USA; [2]Biochemistry, Fels Institute, Temple University, Philadelphia, PA, USA; [3]Nephrology & Hypertension Institute, Sheba Medical Center, Tel-Aviv University, Tel-Aviv, Israel.*

OBJECTIVE: To examine the expressional level of growth arrest- and DNA damage-inducible genes and the downstream signal pathways activation in preecalmpsia

METHODS: Placenta tissue samples from 20 patients with pre-eclamspia in the third trimester (study group) and 20 healthy patients (control group) were freshly collected and preserved respectively in RNAlater reagent and -80°C. Both total RNA and total protein were isolated from placental tissue. Total RNA was reversely transcribed to c-DNA. The mRNA levels of Gadd45a, Gadd45b and Gadd45g were detected with a probe-specific realtime quantitative PCR assay using the RT products as templates. ß-actin was applied in real-time PCR as a reference gene to determine the relative expressional levels of Gadd45 genes. Protein levels of Gadd45a and MAPK signal pathway genes--phospo-p38, phospo-JNK, and phospo-Mkk3/Mkk6 were assessed by western blot with specific antibodies to these genes using ß-actin, total P38, total JNK and total MKK3 proteins as loading control.

RESULTS: With realtime quantitative RT-PCR assay, the average relative expression level of Gadd45a mRNA is 2.3 fold higher in placentas of preeclamptic patients when compared to normal cases($n=20$, $p<0.001$). Both the Gadd45b mRNA and Gadd45g mRNA levels are increased in preeclamptic cases but are not statistically significant. The protein level of Gadd45a was higher in placentas of preeclamptic patients by western blot, which was consistent with results found in Gadd45a mRNA. Phosphorylated P38 was found with western blot in preeclamptic cases but not in normal placentas. While there was no phosphorylation difference of JNK between preeclamptics and controls, P38 phosphorylation was confirmed by western blot detection of phosporylation of MKK3/MKK6, an upstream signal regulating the activation of P38. MKK3 activation was found in placentas of preeclamptic cases but not in the control group.

CONCLUSIONS: Since P38 activation plays an important role in inflammation, immunological occurrence and the balance between survival and apoptosis, environmental stress may contribute to the pathological changes in preeclampsia by activating the P38 signal pathway through Gadd45a one of the stress sensing genes.

744

Differential Regulation of Gelatinase Expression by Human Decidual Cells and Its Implications in Preeclampsia. Charles J Lockwood,* Ceyda Oner, Umit Kaysli, Lynn F Buchwalder, Graciela Krikun,* Edward Funai, Frederick Schatz.* *Obstetrics/Gynecology & Repro. Sci, Yale University, New Haven, CT, USA.*

Objective: Cytotrophoblasts (CT) invade the decidua via CT-expressed integrin-binding to basement membrane (BM) proteins of the peri-decidual cell (DC) extracellular matrix (ECM) followed by their proteolysis. Shallow CT invasion impairs vascular transformation to reduce uteroplacental flow and induce placental hypoxia. Decidual interlelukin-1beta (IL-1) and tumor necrosis factor-alpha (TNF) expression have been linked to preeclampsia (PE). Aberrant cytokine-induced DC-expressed gelatinases A and B may preferentially degrade BM proteins in the decidual ECM and interfere with sequential CT invasion of the decidua. We compared gelatinase A and B expression in normal versus PE decidua and assessed the effects of IL-1 and TNF on gelatinase A and B expression in first trimester DCs.

Methods: Immunohistochemistochemistry (IHC) for gelatinase A and B was performed on specimens of normal decidua (n=4) versus PE (n=5) decidua. Confluent leukocyte-free first trimester DCs were primed with 10^{-8} M E2 + 10^{-7} M medroxyprogesterone acetate (MPA) then switched to a defined medium (DM) with E2 + MPA +/- 1 ng/ml IL-1 or TNF. Secreted gelatinase A and B levels were measured by ELISA and substrate gelatin zymography. Quantitative RT-PCR assessed gelatinase A and B mRNA levels.

Results: Gelatinase B levels, by IHC, were higher in the decidua of PE versus normal women ($p<0.05$), whereas gelatinase A levels were similar in both groups. In first trimester DCs (mean ± SEM, n=8), basal gelatinase A levels (1.7 ± 0.37 ng/ml/ug cell protein) exceeded gelatinase B levels (0.42 ± 0.15 pg/ml/ug protein) by about 3 logs. Exogenous IL-1 and TNF up-regulated gelatinase B output to 1148 ± 516-fold and 122 ± 42-fold ($p<0.05$), respectively, with concentration–dependent effects evident between 0.01 to 10 ng/ml. Substrate gel zymography confirmed that TNF and IL-1 each increased levels of a 92 kD gelatinase B zone, whereas the 72 kD gelatinase A zone was unaffected by the cytokines. Corresponding to similar changes in protein levels, quantitative RT-PCR found that TNF and IL-1 enhanced gelatinase B, but not A, mRNA levels.

Conclusions: At the human implantation site, constitutive DC-expressed gelatinase A expression and inflammatory cytokine-enhanced gelatinase B may promote PE by disrupting decidual ECM to impair normal stepwise CT invasion.

745

Regulation of Interleukin 6-Expression in Decidual Cells; Implications in Preeclampsia. Charles J Lockwood,* Frank C Yen, Murat Basar, Umit Kaysli, Aydin Arici,* Joseph ST Huang, Graciela Krikun,* Frederick Schatz.* *Obstetrics/Gynecology & Repro. Sciences, Yale University, New Haven, CT, USA.*

Objectives: Levels of the multifunctional cytokine, interleukin-6 (IL-6), are elevated in plasma of patients with preeclampsia (PE). Paradoxically, placental IL-6 production and amniotic fluid IL-6 levels are lower in patients with PE than in gestational age-matched controls. The current study evaluated IL-6 levels in normal versus PE decidua by immunohistochemistochemistry (IHC). The association of enhanced thrombin, interlelukin-1beta (IL-1) and tumor necrosis factor-alpha (TNF) with PE prompted assessment of effects of these cytokines on IL-6 expression in first trimester decidual cells (DCs).

Methods: IHC staining for IL-6 was performed on the decidua basalis of 4 specimens of normal versus 6 specimens of PE placentas. Isolated first trimester DCs were purified to near homogeneity, passaged until >99% free of CD45+ cells by FACS, then primed with 10^{-8} M E2 + 10^{-7} M medroxyprogesterone acetate (MPA) to mimic the pregnant steroid milieu. Confluent DCs were switched to a defined medium (DM) with E2 + MPA +/- IL-1 or TNF or thrombin. IL-6 levels in conditioned DM were measured by ELISA and Western blotting. Quantitative RT-PCR assessed IL-6 mRNA levels.

Results: IHC revealed significantly higher IL-6 HSCORE values in DCs from PE versus control specimens ($p<0.05$). In cultured first trimester DCs, ELISA measurements indicated that basal IL-6 levels in conditioned DM were 0.27± 0.10 ng/ml/ug cell protein [mean ± SEM, n=8]. The addition of 2.5 U/ml of thrombin or 1.0 ng/ml of TNF or IL-1 elevated IL-6 output by 4.1± 1.3-fold, 17.5 ± 5.5-fold and 1,164 ± 283-fold, respectively, ($p<0.05$). Western blotting confirmed the ELISA results by demonstrating each cytokine displayed a clear order of potency in increasing the magnitude of the doublet IL-6 molecule with thrombin < TNF < IL-1. Quantitative RT-PCR demonstrated corresponding changes in IL-6 mRNA levels as found for the ELISA measurements.

Conclusions: The current study suggests that DCs are a likely source of the characteristic elevated circulating IL-6 levels during PE. IL-6 alters endothelial cell permeability, inhibits vascular prostacyclin production and impairs endothelial vascular function suggesting that elevated plasma IL-6 levels contributes to the maternal vascular dysfunction of PE.

746

VEGF Inhibits ATP-Induced $[Ca^{2+}]_i$ and NO Production in Endothelium of Intact Uterine Arteries. Fu-Xian Yi, Ronald R Magness,* Ian M Bird.* *Perinatal Research Laboratories, University of Wisconsin-Madison, Madison, WI, USA.*

During pregnancy, the uterine artery (UA) shows increased vasodilation in response to a number of agonists, which in turn contributes to the increase in blood flow necessary to meet the needs of the growing fetus. Recent reports suggest that the circulating levels of VEGF are elevated significantly in both the placenta and circulation of patients with preeclampsia. Excessive VEGF has been considered as a key factor in the pathogenesis of preeclampsia, but the mechanisms remains controversial. Our previous studies have shown that in ovine uterine artery endothelial cells (UAEC, passage 4) from nonpregnant (NP-) and pregnant (P) late term ewes, ATP continuously induced $[Ca^{2+}]$i oscillation and multiple $[Ca^{2+}]_i$ bursts and associated NO production in individual cells over 30 min, and this response is greater in P-UAEC vs NP-UAEC. In this study, we examined whether pre-exposure to VEGF has any effect on this ATP-induced Ca^{2+} responses and associated NO production. We have found that VEGF (10ng/ml) pretreatment does indeed inhibit the multiple Ca^{2+} bursts seen in P-UAEC on subsequent challenge with 100 uM ATP to the level more typical of NP-UAEC without pretreatment, while the less responsive NP-UAEC was not effected by VEGF pretreatment. In order to move more closely to the in vivo situation, we further examined the effect of VEGF on ATP-induced Ca^{2+} and NO production in intact uterine artery endothelium (UAE) on the vessel surface. Using fluorescent microscopy we simultaneously monitored NO production (DAF as probe) and $[Ca^{2+}]_i$ (fura 2 as indicator) in individual endothelial cells of intact UA from ewes. Consistent with UAEC, ATP (100 uM) stimulated more NO in P-UAE > NP-UAE. ATP not only induced a rapid initial robust Ca^{2+} peak but the number of subsequent bursts over 30 minutes was P-UA > NP-UA. VEGF (10 ng/ml) alone stimulated much smaller increase in $[Ca^{2+}]_i$ and NO production compared to ATP, but pretreatment with VEGF for 30 min significant inhibited subsequent ATP-induced $[Ca^{2+}]_i$ response and NO production; this inhibition was more significant in P-UAE than NP-UAE. Conclusions: VEGF pretreatment inhibits ATP-induced Ca^{2+} response and subsequent NO production. These data further validate the UAEC as a valuable cell model and suggest a novel mechanism for how VEGF may contribute to endothelial dysfunction in preeclampsia in vivo. Supported by NIH H050578, HL64601, HL079020, HL 49210, and HD38843.

747

Nicotine and Cotinine Decrease Soluble fms-Like Tyrosine Kinase (sFlt1) and Soluble Endoglin (sEng) Secretion by Human Trophoblast Cells. Anna Tropea,[1] Fiorella Miceli,[1] Francesca Minici,[1] Mariateresa Orlando,[1] Maria F Gangale,[1] Federica Romani,[1] Federica Tiberi,[2] Stefania Catino,[3] Roberta Nestorini,[1] Antonio Lanzone,[3] Rosanna Apa.*[1] *[1]Cattedra di Fisiopatologia della Riproduzione Umana, Università Cattolica del Sacro Cuore, Roma, Italy; [2]Istituto Scientifico Internazionale "Paolo VI", Università Cattolica del Sacro Cuore, Roma, Italy; [3]Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina, EN, Italy.*

Objective: Maternal smoking is associated with a variety of adverse pregnancy outcomes, nevertheless paradoxically women who smoke during pregnancy have a reduced risk of preeclampsia.

Since for this "protective" effect the biologic mechanism is unknown, we examined whether nicotine and its major metabolite cotinine might affect placental secretion of soluble fms-like tyrosine kinase (sFlt1) and soluble endoglin (sEng), both antiangiogenic proteins recently related to preeclampsia. Indeed, excess circulating sFlt1 and sEng of placental origin seems to contribute to the widespread endothelial dysfunction leading to preeclampsia.

Methods: Placentas were collected at the time of elective cesarian sections at term of uncomplicated pregnancies. After their purification, trophoblast cells were cultured for 24h with medium alone (control), or with $CoCl_2$ (chemical hypoxia) 10 μM, or in presence of increasing concentrations (from 10^{-11} to 10^{-6}M) of nicotine or cotinine. In the culture medium sFlt1 and sEng secretion was assayed by ELISA.
Results: As expected, $CoCl_2$ significantly increased both sFlt1 and sEng secretion by trophoblast cells. Conversely, in the same cells all tested doses of nicotine and cotinine were able to significantly decrease both sFlt1 and sEng production.
Conclusions: We demonstrated the ability for nicotine and cotinine to decrease placental secretion of sFlt1 and sEng. This effect could contribute to explain the paradoxical "protective" effect of maternal smoking on preeclampsia.

748

Pentraxin 3 (PTX3) in Maternal and Placental Compartments of Pregnancies Complicated by Preeclampsia and Intrauterine Growth Restriction (IUGR). V Cozzi,[1] C Garlanda,[2] M Nebuloni,[3] V Maina,[2] S Calabrese,[1] A Martinelli,[1] G Pardi,[1] Irene Cetin.*[1] *[1]IRCCS F Policlinico, Mangiagalli, Regina Elena, Inst Obst Gyn, Milan, Italy; [2]Res Lab Imm Infl, Inst Humanitas, Milan, Italy; [3]Pathol Unit Sacco, Dept Clin Sciences, Milan, Italy.*
***Hypothesis**:* Endothelial dysfunction typical of preeeclampsia (PE) is the result of an excessive maternal inflammatory response to pregnancy. PE can occur alone or together with IUGR. PTX3 is increased in myocardial infarction and correlates with disease activity. We have investigated PTX3 in maternal and placental compartments in PE and IUGR pregnancies.
Methods: Maternal blood samples were collected during the III trimester in 33 PE, 24 IUGR and 31 normal pregnancies. Plasma PTX-3 was determined by ELISA. Pattern and site of expression of PTX3 was studied by immunohistochemistry (IHC) on placenta, decidual bed and maternal peritoneum of normal and complicated pregnancies collected at the time of cesarean section.
Results: IUGR and PE mothers were significantly older and had significantly higher BMI than normal pregnancies. PE and IUGR pregnancies delivered earlier because of fetal or maternal indications; their fetal and placental weights were significantly lower than normal pregnancies. PE and IUGR pregnancies showed significantly higher maternal PTX3 levels vs normal pregnancies (19.6±23.1 and 7.8±12.4 vs 2.8±2.3 ng/ml; $p<0.001$ and $p<0.05$, respectively). IUGR showed significantly lower levels than PE ($p<0.05$). Dividing the PE group for clinical severity in mild PE (n=11), severe pure PE (n= 10) and PE complicated by IUGR (n=12), severe pure PE revealed higher levels than the other groups though not significantly different. However, severe pure PE PTX3 levels were significantly higher than pure IUGR (24.1±32.2 vs 7.8±12.4 ng/ml; $p<0.05$). IHC on placenta and decidual bed biopsies showed similar expression in pathologic and normal cases. Maternal peritoneum expressed a significantly higher signal in the endothelium of PE vs normal pregnancies.
Conclusion: We report elevated maternal levels of PTX3 in PE and IUGR pregnancies with higher PTX3 levels in pure severe PE compared to the pure IUGR phenotype. PTX3 increase may represent the expression of endothelial systemic damage on the maternal side, typical of PE, rather than of endothelial local damage on the feto-maternal interface, as suggested by ICH showing high expression on the PE endothelial peritoneum but no differences in decidua and placenta immunostaining.

749

Activated TGFβ Signaling Regulates sFlt-1 Expression in IUGR Pregnancies. Ori Nevo, Jing Xu, Isabella Caniggia.* *Ob/Gyn, Mount Sinai Hospital, SLRI, University of Toronto, Toronto, ON, Canada.*
Background: Elevated sFlt-1 expression found in preeclamptic and IUGR pregnancies have been implicated in the development of the maternal endothelial dysfunction typical of preeclampsia. However, little is known about the mechanisms that control sFlt-1 expression. Recently, we demonstrated that during normal placentation, oxygen is a critical regulator of sFlt-1 expression. In addition, we reported that preeclampsia is associated with placental hypoxia and altered TGFβ signaling. Thus, it is plausible that additional factors beside oxygen, such as transforming growth factor β (TGFβ) molecules, may contribute to the high levels of sFlt-1. Since sFlt-1 expression is elevated in severe IUGR placentae, the **objectives** of the present study were: 1) to examine the expression/activation of Smad2 and 3 (R-Smad) and inhibitory Smad7 (I-Smad) in normal and IUGR pregnancies; and 2) to explore the possibility that TGFβ1/3 signaling via Smads regulate sFlt-1 expression. **Methods:** Early onset (28-33 wks, n=13) severe IUGR with abnormal umbilical and uterine artery Doppler and age-matched normal control placental tissues were collected. First trimester villous explants and BeWo and JEG-3 choriocarcinoma cell lines were cultured in standard conditions in the presence or absence of TGFβ1 or TGFβ3 (5ng/mL) for 24 hours. Message levels of sFlt-1 were measured by quantitative real-time PCR using specific TaqMan primers and probe. Protein expression of sFlt-1 was measured by Western blot using a polyclonal antibody against sFlt-1. In addition, protein lysates from placental tissues, explants and cell lines were analyzed with antibodies that recognize human Smad2/3, Smad7, phospho-Smad2 and phospho-Smad3. **Results:** Phosphorylated Smad2 but not Smad3 was markedly increased in severe IUGR placentae compared to control tissue, while a decreased in inhibitory Smad7 content was observed in IUGR placentae. Exposure of both villous explants and BeWo cells to TGFβ1 and TGFβ3 resulted in increased protein expression of phospho-Smad2 and 3, which was associated with a significant increase in sFlt-1 mRNA and protein levels. In contrast, JEG-3 cells responded to TGFβ treatment only by increasing phospho-Smad2, but not phospho-Smad3 or sFlt-1 mRNA and protein. **Conclusion:** These data suggest that an activation of the TGFβ signaling pathway may in part be responsible for the increased sFlt-1 expression found in both preeclampsia and severe IUGR pregnancies. (Supported by CIHR and OWH/IGH).

750

The Effect of Over-Expression of sFlt-1 in Pregnant Mice on Blood Pressure of the Offspring. Fangxian Lu, Egle Bytautiene, Esther H Tamayo, Garland D Anderson, Gary Hankins,* Monica Longo, George R Saade. *Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, TX, USA.*
Objective: sFlt-1 has been implicated in the etiology of preeclampsia, and over-expression of sFlt-1 in rodents results in a preeclampsia-like condition. Our objective was to determine if fetal programming of adult blood pressure is altered in a previously characterized mouse model of preeclampsia induced by sFlt-1.
Methods: As previously established, CD-1 mothers at day 8 of gestation were injected with an adenovirus carrying Flt (1-3) [AdFlt (1-3); 10^9PFU] or with an adenovirus carrying mFc as control (10^9PFU). The resulting pups were followed until 3 or 6 months of age (average life span 1.5 years), at which time blood pressure (BP) catheters were inserted through the left carotid artery into the aortic arch and connected to a telemetric transmitter. BP was recorded continuously for 6 days in the conscious unrestrained offspring. One-way ANOVA followed by Newman-Keuls post hoc test were used for statistical analysis ($p<0.05$).
Results: At 3 months of age, mean BP was significantly higher during the first 3 days of measurement in the offspring born to sFlt-1-treated mothers (D1: 138.93±2.57 and D3:119.05±1.52mmHg) compared with the control (D1:124.62±4.20 and D3:109.94±2.4mmHg). However, mean BP at 6 months of age was significantly and consistently higher during the entire measurement period in offspring born to sFlt-1-treated mothers (D1: 146.33±4.98 and D6:136.54±2.17mmHg) compared with 6 moths old offspring born to mFc-treated mother (D1:120.76±2.88 and D6:113.54±2.17mmHg). In addition, in the offspring born to sFlt-1-treated mother, BP in 6 month's old offspring was significantly higher than in 3 month' s old. However, there was no significant difference in BP at 3 and 6 months in the offspring born to mFc-treated mother.
Conclusions: Over-expression of sFlt-1 in the mother leads to hypertension in the offspring later in life. Moreover, the hypertension worsens with age. Our findings highlight the role of the intrauterine environment in the developmental origin of adult disease, and the impact of preeclampsia on future health of the offspring.

751

Pre-Pregnant Prediction of Recurrent Hypertensive Complications and Fetal Growth Restriction in a Subsequent Pregnancy. Simone Sep,[1,2] Luc Smits,[1] Marc Spaanderman,[3] Martin Prins,[1] Louis Peeters.*[2] *[1]Epidemiology, Maastricht University, Maastricht, Netherlands; [2]Obstetrics and Gynecology, University Hospital Maastricht, Maastricht, Netherlands; [3]Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, Netherlands.*
Objective: Former preeclamptics have a 20-25% risk of recurrent disease in a next pregnancy. In this study we developed prediction models to forecast individual recurrence risk in women with a history of severe preeclampsia (PE) and/or HELLP syndrome (delivery <34 weeks).

Methods: We measured a wide range of cardiovascular, hemostatic, immunologic, and metabolic variables in 150 women with a recent history (6-12 months earlier) of severe PE or HELLP syndrome, and who completed a subsequent ongoing pregnancy. Using the pre-pregnant data and the outcome data of the next pregnancy, we developed models to predict recurrent PE/HELLP and severe FGR (birth weight < 1500 g) in that next pregnancy using binary logistic regression analysis. By Receiver Operating Characteristic (ROC) analysis, we assessed the predictive capacity of the obtained models.
Results: Severe PE/HELLP syndrome recurred in 11 (7%), and severe FGR occurred in 7 (5%) next pregnancies. The models predicting these two outcomes (models I and II, resp.) included the following variables: pre-pregnant HDL-cholesterol [I, II], mean arterial pressure (MAP) [I, II], vena cava collapsibility index [II], and the circulating level of ALAT liver-enzymes [II]. The discriminating capacities of the models are illustrated in the figures below.
Conclusion: The two multi-variable prediction models, developed for women with a history of severe PE/HELLP identified, with clinically relevant predictive capacity, those women at increased risk for severe recurrent disease, and those at increased risk for severe FGR in their next pregnancy. Prospective evaluation of the performance of the models in an independent population of former preeclamptics is needed to assess external validity.

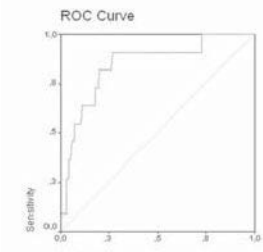


Fig. 1: ROC-curve for recurrent severe PE/HELLP syndrome. AUC = 0.84, 95%CI(0.72-0.97). With a cut-off value of ≥7% (predicted probability), Se is 91% (57%-100%); Sp 73% (64%-80%); +PV 21% (11%-35%); -PV 99% (94%-100%).

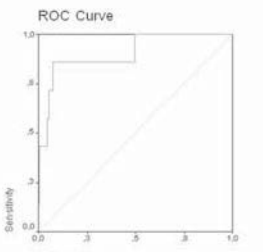
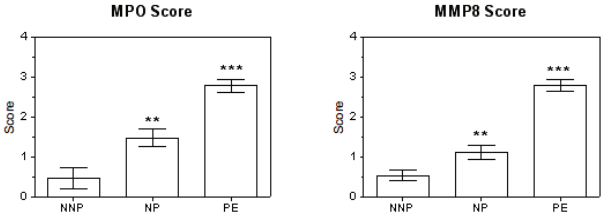


Fig. 2: ROC-curve for severe FGR. AUC = 0.89, 95%CI(0.76-1.00). With a cut-off value of ≥8% (predicted probability), Se is 86% (42%-99%); Sp 89% (82%-93%); +PV 27 % (12%-50%); -PV 99% (95%-100%).

752

The Neutrophil Products, Myeloperoxidase and Matrix Metalloproteinase 8, Are Increased in Systemic Vasculature of Preeclamptic Women. Juhi Shukla, Sonya Washington, Scott W Walsh.* *OBGYN, Physiology, Virginia Commonwealth Univ, Richmond, VA, USA.*
Neutrophils infiltrate systemic vascular tissue in women with preeclampsia. Neutrophils produce reactive oxygen species, inflammatory cytokines, and other compounds that can be toxic to tissue. For example, myeloperoxidase (MPO) could cause oxidative stress by producing hypochlorous acid, and matrix metalloproteinase 8 (MMP8) could cause a loss of cell integrity by degrading collagen. HYPOTHESIS: Systemic vascular tissue of preeclamptic women will have a significant presence of MPO and MMP8 as a result of neutrophil infiltration. METHODS: Subcutaneous fat, which is highly vascularized, was obtained at abdominal surgery from 4 normal non-pregnant (NNP), 5 normal pregnant (NP) and 5 preeclamptic (PE) women. Formalin fixed, paraffin embedded 8 µm sections of fat biopsies were stained using immunohistochemistry with specific antibodies for MPO and MMP8. Data were evaluated for intensity of vessel staining by visual score (0-4), density of staining using image analysis software, and % vessels with neutrophil staining, diffuse staining and vascular smooth muscle staining. Resistance-sized vessels (10-200 µm) were evaluated. RESULTS: Intensity of vessel staining assessed by visual score was significantly greater for PE than NP or NNP (Fig). Density measurements were highly correlated with visual score for both MPO and MMP8 (r = 0.98, r = 0.99). The % vessels with neutrophils stained for MPO and MMP8 was significantly greater (P<0.001) for PE than NP or NNP: MPO (88±5 vs. 66±4 vs. 27±20%); MMP8 (88±3 vs. 54±16 vs. 32±9%), as were % vessels with diffuse staining: MPO (79±5 vs. 44±13 vs. 14±14%); MMP8 (80±13 vs. 38±14 vs. 21±9%), and % vessels with vascular smooth muscle staining: MPO (49±7 vs. 18±14 vs. 4±6%); MMP8 (55±14 vs. 10±6 vs. 2±2%). CONCLUSIONS: In women with PE, there is increased presence of MPO and MMP8 in systemic vasculature as a result of neutrophil infiltration. SPECULATION: MMP8 by causing cellular matrix breakdown could account for vascular inflammation, and MPO by inactivating nitric oxide could be responsible for vasoconstriction leading to hypertension in PE. HL069851.



753

Tumor Necrosis Factor Alpha in Obesity and Preeclampsia. Sandra Founds,[1] Robert Powers,[*2] Patrick Thelma,[1] James Roberts.[*2] *[1]School of Nursing, University of Pittsburgh, Pittsburgh, PA, USA; [2]Magee-Womens Research Institute and Dept Ob/Gyn & Reprod Sciences, University of Pittsburgh, USA.*
Objective: Tumor necrosis factor alpha (TNFa) is higher in preeclampsia and is proposed to be a mediator of inflammatory processes associated with endothelial dysfunction. TNFa is also elevated in obese nonpregnant individuals. Little is known about the relationship between obesity and TNFa in preeclampsia. We asked if increased TNFa explains the increased frequency of preeclampsia with obesity. Hypotheses: TNFa is 1)higher in obese than lean 2)higher in preeclamptic than control 3)higher in obese control than lean control 4)higher in lean preeclamptic than obese control 5)higher in obese preeclamptic than lean preeclamptic participants.
Methods: Nested case-control study of lean (prepregnancy BMI 19-24.9 kg/m^2) and obese (prepregnancy BMI≥ 30 kg/m^2) nulliparous uncomplicated control and preeclamptic women. Total TNFa was measured by ELISA in EDTA plasma samples collected at admission for delivery. Analyses included univariate ANOVA and general linear modeling with Bonferroni adjustment for multiple comparisons; significance accepted at p<0.05.
Results: Gestational age was lower in preeclamptic than control participants (p<0.0001), but no different between lean and obese control (p=0.81) or between lean and obese preeclamptic participants (p=0.71). By design, there was no difference in BMI between lean preeclamptic and lean control participants (p=0.87), or between obese preeclamptic and obese controls (p=0.59). There was no difference in TNFa by BMI (p=0.27). TNFa was higher in preeclamptic than control participants (p=0.012). TNFa was not higher in obese control than lean control (p=1.0), was higher in lean preeclamptic than obese control (p=.04), was not higher in obese preeclamptic than lean preeclamptic participants (p=.31). There was no interaction between diagnosis and BMI (p=0.09). (Table)
Conclusions: As in previous studies, TNFa was higher in preeclamptic than control participants. However, our hypothesis that TNFa would be higher in obese than lean preeclamptic women was not supported. Increased TNFa does not explain the increased frequency of preeclampsia in obesity.

TNFa Data

	Lean Control	Obese Control	Lean PE	Obese PE
Prepregnancy BMI	21.5±0.3	36.4±1.0	21.7±0.3	35.9±0.9
TNFa (pg/ml)	1.0±0.1	1.0±0.1	1.4±0.1	1.2±0.1

Data: mean±SE

754

Do Differences in Antiangiogenic Factors Precede the Development of Preeclampsia in High-Risk Women? Robert W Powers,[*1] Arundhathi Jeyabalan,[*1] for the NICHD MFMU Network.[2] *[1]Obstetrics & Gynecology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Bethesda, MD, USA.*
Objective: Alterations in the maternal circulating concentrations of the antiangiogenic factors soluble fms-like tyrosine kinase 1 (sFlt1) and endoglin and the angiogenic growth factor placental growth factor (PlGF) have been shown to precede the development of preeclampsia by weeks to months in healthy low-risk women. The objective of this study was to investigate whether similar alterations occur in women at high-risk of developing preeclampsia including women with chronic hypertension, diabetes, multifetal gestation, and previous preeclampsia.

Study Design: This is a secondary analysis of the NICHD MFMU trial of aspirin (ASA) to prevent preeclampsia in high-risk pregnancies. sFlt1, endoglin and PlGF were measured in serum samples collected from 993 women at randomization (average=19.3, range 7.6 to 26.9 weeks gestation). The inter-assay coefficient of variation for each analyte was sFlt1=10%, endoglin=11%, and PlGF=7%. Data are presented as mean±standard deviation, and were analyzed by Wilcoxon Rank Sum test and logistic regression adjusted for gestational age at sample collection, maternal age, smoking, race and BMI.
Results: At baseline, the serum concentrations of sFlt1, endoglin and PlGF were significantly elevated in women with multifetal gestation compared with women with diabetes, chronic hypertension or previous preeclampsia (all p<0.0001). In multivariable analysis, only in women with multifetal gestations were sFlt1 and endoglin associated with an increased the risk of preeclampsia (OR 1.19, 95% CI 1.06-1.34 and OR 1.19, 95% CI 1.06-1.33, respectively).
Conclusion: Compared with other high-risk groups, sFlt1, endoglin and PlGF are elevated in women with multifetal gestation. Elevations in the antiangiogenic factors sFlt1 and endoglin precede the development of preeclampsia in women with multifetal gestation.

	Insulin Dependent Diabetes		Chronic Hypertension	
	Normal (n=148)	Preeclampsia (n=46)	Normal (n=235)	Preeclampsia (n=78)
sFlt1 (ng/ml)	3.6±2.0	3.8±2.3	3.7±3.7	3.8±2.9
Endoglin (ng/ml)	5.3±1.6	5.8±2.3	5.6±5.7	6.1±3.6
PlGF (pg/ml)	151.1±144.6	156.7±147.5	222.5±236.7	191.6±154.2
	Multifetal Gestation		Previous preeclampsia	
	Normal (n=195)	Preeclampsia (n=39)	Normal (n=202)	Preeclampsia (n=50)
sFlt1 (ng/ml)	6.0±2.5	7.3±5.4	3.3±1.8	3.2±1.7
Endoglin (ng/ml)	7.0±2.7	8.5±4.5	5.5±4.9	5.6±2.7
PlGF (pg/ml)	554.7±388.1	386.1±318.2	231.6±218.9	191.4±159.2

755

ADMA Is Higher and PlGF Is Lower in Obese Women Who Later Develop Preeclampsia Compared to Similarly Obese Women with Uncomplicated Pregnancies. Robert W Powers,[*1,2] Michael P Frank,[1] Ashi Daftary,[1,2] Roberta B Ness,[1,3] James M Roberts.[*1,2,3] *[1]Magee-Womens Research Institute; [2]Obstetrics & Gynecology; [3]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA.*
Objective: Obesity increases risk for preeclampsia by mechanisms that are not clear. Endothelial dysfunction, central to the pathophysiology of preeclampsia, is common in obesity. Alterations in angiogenic growth factors may contribute to this dysfunction. Since these factors exert their activity in part through a nitric oxide (NO) dependent mechanism alterations in the endogenous inhibitor of NO synthase asymmetric dimethylarginine (ADMA) may contribute to this dysfunction. We tested whether alterations in ADMA and placental growth factor (PlGF) are present in obese compared to lean women at mid-pregnancy and are further altered in women who develop preeclampsia.
Study Design: This was a case-control study of 29 lean (lean control, BMI=20.9±1.6kg/m2) and 14 obese pregnant women (obese control, BMI=34.8±3.7kg/m2) who had uncomplicated pregnancies, 10 lean (lean preeclampsia, BMI=21.7±1.1kg/m2) and 10 obese pregnant women (obese preeclampsia, BMI=34.9±2.9kg/m2) who developed preeclampsia. ADMA and PlGF were measured at 18.2±2.1 weeks gestation. Data are mean±SD, and statistical analysis was by one-way ANOVA with statistical significance accepted at p<0.05.
Results: ADMA in mid-pregnancy was higher in obese (0.40±0.06µmol/L) compared to lean controls (0.32±0.07µmol/L, p<0.01), and ADMA was further elevated in both lean and obese women who later developed preeclampsia (0.45±0.07 and 0.48±0.08µmol/L respectively, p<0.05) compared to both lean and obese controls. Conversely, the concentration of PlGF was lower in obese (119.5±35.9pg/ml) compared to lean controls (166.1±81.0pg/ml, p<0.05). PlGF was also lower in lean women who later developed preeclampsia (121.2±25.5pg/ml, p<0.05) compared to lean controls, and lowest among the obese women who later developed preeclampsia (65.5±48.7pg/ml, p<0.05). However, there was no correlation between ADMA and PlGF between groups (r=0.14, p=0.38).
Conclusion: ADMA is higher and PlGF lower at mid-pregnancy in obese compared to lean pregnant women, and both groups women who later develop preeclampsia have higher ADMA and lower PlGF than comparable women with uncomplicated pregnancy outcome. Alterations in ADMA and PlGF may contribute to the mechanism by which obesity increases the risk of preeclampsia.
Funded by NIH-5MO1-RR00056 and NIH-2PO1-HD30367.

756

Evidence of Endothelial Dysfunction in Preeclampsia Identifies Increased Risk of Adverse Pregnancy Outcome. Robert W Powers,[*1,2] Janet M Catov,[1,3] Kristine Y Lain,[*1,2] Marcia J Gallaher,[1] Ashi Daftary,[1,2] James M Roberts.[*1,2,3] *[1]Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetrics & Gynecology, University of Pittsburgh, Pittsburgh, PA, USA; [3]Epidemiology, Pittsburgh, PA, USA.*
Background: Endothelial dysfunction is central to the pathophysiology of the pregnancy syndrome preeclampsia.
Objective: We tested the hypothesis that elevated cellular fibronectin (cFN), a marker of endothelial injury, would be associated with a higher frequency of preterm delivery or SGA infants compared to women with hypertension, and proteinuria with or without hyperuricemia.
Methods: The concentration of plasma cFN in samples collected at admission for delivery was measured by ELISA in 968 women. Definitions of clinical variables include: gestational hypertension (≥140mmHg SBP or 90mmHg DBP), proteinuria (2+, >300mg/24hr, or >0.3 protein/creatinine), gestational hyperuricemia (>1sd gestational age), and elevated cFN (highest quartile). Outcomes were preterm birth (<37 weeks) and small for gestational age (SGA) infants (<10% corrected for gestational age, race and sex). Logistic regression was utilized to estimate risk for preterm, SGA, or both preterm and SGA, after adjustment for BMI and smoking.
Results: Elevated cFN, in the presence of hypertension and proteinuria (HP) was associated with higher risk for preterm delivery (OR 2.5, CI 1.2-5.1), SGA (OR 2.8, CI 1.1-7.2), and the risk for both preterm and SGA was 5.4-fold (CI 2.4-17.6) higher than HP. We have previously shown that the presence of elevated uric acid in preeclampsia (HPU) is associated with higher frequency of preterm birth and SGA compared to HP without elevated uric acid. Elevated cFN in HPU women was associated with a further increase of SGA (OR 2.7 CI 1.0-7.1). Infants born both preterm and SGA was 4.2-fold (CI 1.2-14.3) more common with high cFN in HPU. About 65% of women with preeclampsia, with or without hyperuricemia, also had elevated cFN. Among normotensive women, those with elevated cFN had no increased risk for any adverse infant outcomes.
Conclusions: Elevated cFN is prevalent among women with preeclampsia and identifies increased risk of preterm birth and SGA. However, isolated evidence of endothelial dysfunction at delivery is not associated with an increased risk of an adverse pregnancy outcome.
Funded in part by National Institutes of Health NIH-5MO1-RR00056 and NIH-2PO1-HD30367.

757

Prepregnancy Plasma Volume Predicts Early Pregnancy Plasma Volume. Ira Bernstein,[*1] Adrienne Schonberg,[1] Beth Bouchard,[2] Alan Segal,[3] Robert Shapiro.[4] *[1]OB/GYN, Univ of VT, Burlington, VT, USA; [2]Biochemistry, Univ of VT, Burlington, VT, USA; [3]Medicine, Univ of VT, Burlington, VT, USA; [4]Neurology, Univ of VT, Burlington, VT, USA.*
BACKGROUND: Reduced maternal plasma volume in pregnancy is associated with adverse pregnancy outcome including fetal growth restriction and preeclampsia. We sought to determine the degree to which maternal plasma volume during pregnancy is dependent on prepregnancy plasma volume.
METHODS: We examined 22 healthy, non-smoking women during the follicular phase of the menstrual cycle (MC) to determine plasma volume employing Evans blue dye dilution after an overnight fast. All women subsequently conceived with singleton viable pregnancies and were re-examined in early pregnancy (84.3 $\pm$ 15 menstrual days) to determine plasma volume using the same technique. We examined plasma volume in pregnancy as a dependent variable employing stepwise linear regression modeling. Independent predictors included prepregnancy plasma volume (BPV), prepregnancy body mass index (BMI), body surface area (BSA) and maternal height as well as gestational age at pregnancy assessment. Data is expressed as mean $\pm$ standard deviation.
RESULTS: Subjects were 28.4 $\pm$ 2.8 years old with a BMI of 23.1 $\pm$ 3.2 kg/m^2 at the time of prepregnant studies. Maternal prepregnancy plasma volume was 2,587 $\pm$ 547 mL and increased to 2,912 $\pm$ 582 mL (12.6% increase). This volume expansion was associated with an 8% drop in hematocrit (37.0 $\pm$ 2.4 to 34.1 $\pm$ 2.3 %). Univariate analysis demonstrated significant associations of pregnancy plasma volume to BPV (r=0.89, p<0.001), gestational age (r=0.56, p=0.007), BSA (r=0.53, p=0.01) and height (r= 0.48, p=0.03). Stepwise regression demonstrated a significant association of only BPV to early pregnancy plasma volume with a correlation coefficient or 0.89, p<0.001.

CONCLUSIONS: Early pregnancy plasma volume is strongly associated with prepregnancy plasma volume. Prepregnancy plasma volume accounts for 79% of the variance observed in early pregnancy plasma volume. This observation supports the hypothesis that prepregnancy phenotype contributes to the obstetric complications observed during pregnancy. Observations are continuing to evaluate the relationship of third trimester plasma volume to prepregnancy status. Supported by NIH RO-1 HL 71944.

758

Evaluation of Circulating Acylcarnitine Concentrations during Pregnancy: Uncomplicated, IUGR or Preeclampsia in Relation to β-Oxidation. Rhobert W Evans,[1] Carl A Hubel,[*2] James M Roberts,[*2] Robert W Powers,[*2] Shekhar Mehta,[3] Donald H Chace,[4] Joseph M Quashnock.[4] *[1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Magee Research Institute, Pittsburgh, PA, USA; [3]Biostatistics, University of Pittsburgh, Pittsburgh, PA, USA; [4]Pediatrix Screening, Inc, Bridgeville, PA, USA.*

Objective: Little is known concerning the relevance of acylcarnitine concentrations in adults. The focus of this study was to investigate their serum levels during pregnancy.

Methods: Maternal serum samples collected at term were analyzed by tandem mass spectrometry and represented uncomplicated pregnancies (n=23), IUGR (n=21) and preeclampsia (n=17).

Results: Data for significant differences, analyzed by t-test after adjustment for gestational age, are shown in the table.

Table 1: Mean Acylcarnitine Concentrations (γM)

Analyte	Uncomplicated	PE	p	IUGR	p
C50H	0.0093	0.0105	0.63	0.0154	0.030
C102	0.0088	0.0163	0.039	0.0096	0.79
C101	0.0457	0.0685	0.030	0.0521	0.41
C5dcc/10OH	0.0170	0.0348	0.00035	0.0194	0.43
C14	0.0127	0.0210	0.059	0.0198	0.030
C182	0.0211	0.0361	0.015	0.0185	0.048
C18	0.0157	0.0240	0.040	0.0185	0.031
C161	0.0081	0.0153	0.025	0.0106	0.26

Two acylcarnitines C5OH and C14 were higher in IUGR pregnancies. Conversely six acylcarnitines were elevated in preeclampsia. Grouping of the analytes(C4 + C5; C6 + 8 + 81 +10 + 101 +102; C12 + 121 + 14 + 141 + 142 + 16 + 161 + 18 + 181 + 182) suggest that during preeclampsia the activity of the long chain acyl CoA dehydrogenase is reduced. However, lower carnitine acylcarnitine translocase or carnitine palmitoyl transferase II activity cannot be ruled out. The limitations of the study include the small sample size and the large number of analytes.

Conclusions: The changes in acylcarnitine concentrations in preeclampsia reflect increases of up to 205%, but they remain very low compared to those observed in newborns with inborn errors of metabolism. There may be an impairment in the β-oxidation of long chain fatty acids during preeclampsia but not during IUGR pregnancies.

759

Identification of Proteins Associated with Small for Gestational Age (SGA) in a Subgroup of Preeclampsia Using 2-D DIGE. Marion Blumenstein,[1] Steven Wu,[1] Michael T McMaster,[1,3] Mik Black,[4] Garth JS Cooper,[1] Robyn A North.[2] (SPON: Peter Stone). *[1]School of Biol Sciences, Univ of Auckland, Auckland, New Zealand; [2]Obs & Gyn, Univ of Auckland, New Zealand; [3]Cell & Tissue Biol, Univ of California, San Francisco, USA; [4]Biochemistry, Univ of Otago, Dunedin, New Zealand.*

Background: Preeclampsia (PE) is associated with abnormal placentation, particularly in cases of fetal growth restriction. Placenta derived proteins are secreted into maternal blood and many circulating biomolecules are known to be altered in placental insufficiency. We used Difference in Gel Electrophoresis (DIGE) to identify sets of plasma proteins that may serve as biomarkers. **Objective**: To identify differentially expressed proteins in maternal plasma prior to the onset of PE alone or PE with small for gestational age babies (PE+SGA) compared to uncomplicated pregnancies. **Study Design**: As part of the SCOPE (Screening for Pregnancy Endpoints) study, maternal plasma was collected at 20weeks' gestation and biobanked. A nested case-control study of PE alone (n=8), PE+SGA (n=6) and healthy controls (n=8) was conducted. Plasma was depleted by using a MARS immunoaffinity column (Agilent) for the six most abundant proteins. Pooled plasma from all cases and controls was used for the internal standard (I.S.). Depleted plasma from each case, control and the I.S. was labeled with fluorescent dyes Cy3, Cy5 and Cy2, respectively. Labeled plasma from a case, a control and the I.S. were focused together at pH 4-7, then separated on 8-16% Tris HCl Criterion gels (Biorad). Gels were analyzed by DeCyder v6.5 software (GE) for differential protein expression. Statistical analyses included Student's *t*- and Mann Whitney-tests. False positives were eliminated by applying a false discovery rate (FDR) correction. Nearest shrunken centroid classification was also used. **Results**: In the PE+SGA group, 16 proteins were increased 1.4-10.7 fold (p=0.03 to 0.0003) and two were 1.4 and 2.1 fold decreased compared to healthy controls. In the PE alone group no proteins were found to be significantly different compared to controls. Identification of proteins by mass spectrometry is currently in progress. **Conclusions**: A set of 18 differentially expressed plasma proteins were identified in women at week 20 of pregnancy who later developed PE+SGA. This finding highlights the importance of reduced technical variability of 2D-DIGE compared to standard methods for differential expression studies in complex mixtures such as plasma.

760

Accuracy of Body Mass Index To Predict Preeclampsia: Systematic Review and Bivariate Meta-Analysis. Jeltsje S Cnossen,[1,2] Mariska MG Leeflang,[3] Emmelieke EM de Haan,[1] Ben WJ Mol,[2] Joris AM van der Post,[2] Khalid S Khan,[4] Gerben ter Riet.[2] (SPON: Stephen C Robson). *[1]General Practice, Academic Medical Center, Amsterdam, Netherlands; [2]Obstetrics and Gynecology, Academic Medical Center, Amsterdam, Netherlands; [3]Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam, Netherlands; [4]Obstetrics and Gynecology, Birmingham Women's Hospital, Birmingham, United Kingdom.*

Objective To determine the accuracy of prepregnancy body mass index (BMI) in predicting pre-eclampsia.

Design Systematic review and bivariate meta-analysis to estimate sensitivity and specificity.

Data sources Medline, Embase, Cochrane Library, MEDION, manual searching of reference lists of review articles and eligible primary articles, and contact with experts.

Review methods Reviewers independently selected studies, and extracted data on study characteristics, quality and accuracy. Language restrictions were not applied. Pooled sensitivities and specificities with 95% confidence intervals and a summary Receiver Operating Characteristic (sROC) curve were estimated using the bivariate method for meta-analysis. The potential value of BMI was assessed by combining its predictive capacity for different prevalences of pre-eclampsia and the therapeutic effectiveness (relative risk 0.90) of aspirin.

Results 36 studies, testing 1,699,244 pregnant women (60,621 pre-eclamptic cases), met the selection criteria. The median incidence of pre-eclampsia was 3.9% (IQR 1.4-6.8). The area under the curve was 0.64 with 93% of heterogeneity explained due to threshold differences. Pooled estimates for all studies with a BMI ≥25, were 47% (95% CI 33–61) for sensitivity and 73% (95% CI 64–83) for specificity. For a BMI ≥35 these estimates were 21% (95% CI 14–28) and 93% (95% CI 91–96), respectively. At the upper IQR prevalence limit of 6.8% the positive predictive value of a BMI ≥35 was 18%, whereas at the lower limit of 1.4% this was 4.1%. The number needed to treat to prevent one case of pre-eclampsia without measuring BMI in low risk women was 714 without testing whereas it was 34 at BMI ≥35 among high risk women.

Conclusion A simple non-invasive inexpensive ubiquitous measurement such as BMI can help classify risk of pre-eclampsia in pregnant woman and to tailor their treatment. With high specificity BMI, when normal, will help rule out the risk of pre-eclampsia. When abnormal, decisions for further test, monitoring or treatment can be easily made taking into account the BMI level.

761

Uterine Artery Doppler To Predict Preeclampsia and Fetal Growth Restriction: A Systematic Review and Bivariate Meta-Analysis. Jeltsje S Cnossen,[1,2] R Katie Morris,[3] Gerben ter Riet,[1] Ben WJ Mol,[2] Joris AM van der Post,[2] Arri Coomarasamy,[3] Aeilko H Zwinderman,[4] Stephen C Robson,[*5] Patrick JE Bindels,[1] Jos Kleijnen,[6] Khalid S Khan.[3] *[1]General Practice, AMC, Amsterdam, Netherlands; [2]Obs & Gyn, AMC, Amsterdam, Netherlands; [3]Obs & Gyn, Birmingham Women's Hospital, Birmingham, United Kingdom; [4]Clin Epi & Biostat, AMC, Amsterdam, Netherlands; [5]Obs & Gyn, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; [6]Kleijnen Systematic Reviews Ltd, York, United Kingdom.*

Objective To determine the accuracy and clinical value of uterine artery Doppler indices to predict preeclampsia (PET) and fetal growth restriction (FGR).

Methods Systematic searches in Medline, Embase, Cochrane Library, MEDION (until April 2006), reference lists, and contact with experts. All studies reporting on uterine artery Doppler before the 25th gestational week, and data for a 2x2 table were selected. No language restrictions. Multiple reviewers independently selected studies, extracted data, and assessed study

validity. A bivariate meta-analysis of sensitivity and specificity was conducted when appropriate. The potential value of Doppler was assessed by combining its predictive capacity for different prevalences of PET and FGR and the therapeutic effectiveness of aspirin.

Results There were 74 studies, testing 78,133 women (2,396 cases) for PET and 60 studies testing 40,637 women (4,067 cases) for FGR. For PET, a high resistance index or notching showed the best pooled sensitivity (80%, 95% CI 66-94; pooled specificity 82%, 95% CI 74-91) and the best sROC curve, while pulsatility index with notching showed the best pooled specificity (99%, 95% CI 98-99; pooled sensitivity 22%, 95% CI 16-28). For FGR, resistance index with notching showed the best sROC curve (pooled sensitivity 40%, 95% CI 20-61; pooled specificity 91%, 95% CI 87-95), while the best specificity was achieved by pulsatility index with notching (99%, 95% CI 98-99; sensitivity 12%, 95% CI 8-18). The accuracy did not show variation in clinically relevant subgroups. Numbers of patients needed to be treated (with aspirin therapy) to reduce PET or FGR were much lower with testing than without.

Conclusion Uterine artery Doppler assessment, an increased resistance index or presence of notching in particular, is a useful test to predict PET but has a limited role in the prediction of FGR. Doppler assessment combined with preventive treatment such as aspirin can be useful in high risk women in particular.

762

BMI and Preeclampsia: What Is the Real Risk? Sindhu K Srinivas,[1] Jacob Larkin,[1] Andrea Goldberg,[1] Mary Sammel,[2] Michal A Elovitz.[*1] *[1]OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA.*

Background: Obesity is a known risk factor for cardiovascular disease (CVD). Recent literature suggests that preeclampsia (PEC) specifically preterm PEC, increases a woman's risk for long-term CVD. We sought to determine the relationship between early pregnancy obesity (EP obesity; BMI >=30) and late pregnancy obesity (LP obesity; BMI >=30) on the development of PEC.

Methods: This study was part of a large case control study, *Preeclampsia: Mechanisms and Consequences*. Cases are patients prospectively identified with PEC using maternal criteria. Controls were term deliveries. Demographic, obstetric, prenatal and neonatal information were collected similarly for all cases and controls. Chi square and Fisher's exact tests were used to determine if EP obesity or LP obesity was associated PEC. Associations of interest were adjusted for confounders using multivariable logistic regression.

Results: 216 cases and 213 controls were evaluated. 48% percent of cases and 36% of controls have EP obesity (p=0.009). 70% cases and 57% controls have LP obesity (p=0.007). The presence of chronic hypertension and/or diabetes (CHTN/DM) modifies the effect of EP obesity on the development of PEC after controlling for maternal age, race, gestational age at delivery, and weight gain in pregnancy. In women without CHTN/DM, EP obesity increases the odds for developing preeclampsia by 2.5 ([1.33-4.68], p=0.004) compared to non-obese women. However, in women with CHTN/DM, there was no significant difference in the risk of PEC between obese women compared to non-obese women (OR=0.23 [.038-1.37] p=0.106). Women with both CHTN/DM and EP obesity have an increased odds of developing PEC (OR=5.21 [2.04-13.27], p=0.001) compared to non-obese women without CHTN/DM. LP obesity also has an independent effect on the development of PEC (OR= 1.93[1.07-3.47], p=0.028) after controlling for the above confounders and CHTN/DM.

Conclusion: These studies suggest that EP obesity and LP obesity are each independently associated with the development of PEC. The significant interaction between medical co-morbidities and EP obesity suggests that pre-existing pathways in the mother may be mechanistically involved in the pathogenesis of preeclampsia. Studies to evaluate the mechanism by which obesity modifies PEC disease risk is warranted in order to advance prevention strategies.

763

PAPP-A Concentrations Are Not Higher in Superimposed Preeclampsia. Kristiina Parviainen,[1] James M Roberts.[*1,2] *[1]Division of Maternal-Fetal Medicine; Department of OB/GYN/RS, University of Pittsburgh, Pittsburgh, PA, USA; [2]Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA.*

Objective: Pregnancy-associated plasma protein A (PAPP-A) is a metalloprotease that cleaves insulin-like growth factor binding protein 4, releasing insulin-like growth factor. PAPP-A production occurs in many organs, including the placenta, with serum concentration increasing up to 100-fold in pregnancy. Low PAPP-A concentrations in early pregnancy are associated with adverse pregnancy outcome including aneuploidy and preeclampsia. Outside of pregnancy, PAPP-A is identified in unstable atherosclerotic plaques and predicts adverse outcome in acute coronary syndrome. Some investigators also report higher PAPP-A late in pregnancies complicated by preeclampsia. The association of preeclampsia and cardiovascular disease may suggest a pathophysiologic role for PAPP-A in both diseases. We hypothesized that, among a population of pregnant women with pregestational hypertension, those who developed superimposed preeclampsia (SPE) would have elevated serum PAPP-A concentrations compared with those who did not (CHTN). The specificity of PAPP-A as a marker for acute coronary events, rather than for chronic disease, led us to further hypothesize that these differences would be most pronounced during clinically evident disease.

Methods: We identified samples from 66 women with pregestational hypertension (35 CHTN and 31 SPE) who were recruited for the Prenatal Exposures and Preeclampsia Prevention Project at Magee-Womens Research Institute between 1997 and 2002. A total of 80 samples (22 first trimester and 58 third trimester) were assayed for PAPP-A with a commercially available enzymatically amplified sandwich-type immunoassay (DSL ACTIVE®). The Wilcoxon rank sum test was used to quantitate differences in PAPP-A.

Results: First trimester PAPP-A concentrations were similar for CHTN and SPE [median 0.803mIU/mL (range .09-5.11) vs. 1.01mIU/mL (0.04-2.75), p=.66], as were third trimester concentrations [51.89mIU/ml (8.1-156.6) vs. 45.66mIU/ml (21.11-135.6), p=.96]. Because gestational age at sampling was lower in the SPE group (35.8 wks vs. 38.0 wks, p=.001), we analyzed samples >37 weeks and found no difference between the two groups (p=. 57).

Conclusion: In our population, PAPP-A concentrations in the first and third trimesters did not discriminate women with pregestational hypertension destined to develop superimposed preeclampsia.

764

Platelet Volume Is Directly Associated with Plasma Volume and Inversely Associated with Platelet Concentration Prior to Pregnancy. Cresta Jones,[1] Ira M Bernstein,[*1] Adrienne Schonberg,[1] Beth Bouchard.[2] *[1]Ob/Gyn, University of Vermont, Burlington, VT, USA; [2]Biochemistry, University of Vermont, Burlington, VT, USA.*

Introduction: Preeclampsia has been associated with evidence of increased platelet size, increased platelet activation and low plasma volume during pregnancy. We sought to determine the relationship of platelet volume to both plasma volume and platelet activation prior to a first pregnancy. **Methods:** We examined 14 young healthy nulligravid women. Mean age was 27.1 $\pm$ 4.3 years. BMI was 23.7 $\pm$ 3.2 (kg/m^2). The subjects were predominantly Caucasian (79%, 11/14). Plasma volume was estimated employing Evans blue dilution with multiple post injection samples and corrected for body surface area. Platelet volume (MPV) was estimated by automated analysis. Platelet activation was estimated employing flow-cytometry calculating the percentage of platelets or platelet aggregates demonstrating specific suface antigens: 1) isolated activated platelets (CD63) 2) platelet monocyte aggregates (CD14/CD61) and platelet neutrophil aggregates (CD66b/CD61). Statistical analysis was performed by Pearson correlation coefficient with P<0.05 accepted for significance.**Results** Baseline plasma volume was 3139 $\pm$ 366 mL. Baseline platelet concentration was 235 $\pm$ 71 (K/mL). Mean MPV is 8.8 $\pm$ 1.0 cL. Mean platelet volume was inversely associated with platelet concentraton (r = - 0.68, P<0.001). Platelet volume was significantly and positively associated with plasma volume (corrected for body surface area) (r = 0.69, P=0.006). None of the indices of platelet activation were associated with platelet volume: isolated platelets, (r = -0.12, P = 0.70), platelet monocyte aggregates (r = 0.40, P = 0.16) platelet neutrophil aggregates (r = -0.08, P = 0.80). **Conclusions** Prior to pregnancy mean platelet volume is inversely associated with platelet concentrations. In contrast to the observations made during preeclampsia mean platelet volume is significantly and positively associated with plasma volume. We also observed no significant association of MPV with indices of platelet activation. These findings appear to contrast with the observation made during preeclamptic pregnancy.

765

Prepregnancy Maternal Uric Acid Concentration Correlates with Body Mass Index but Not Renal Function or Plasma Volume. Ira Bernstein,[*1] Adrienne Schonberg,[1] Beth Bouchard,[2] Robert Shapiro,[3] Alan Segal.[4] *[1]OB/GYN, University of VT, Burlington, VT, USA; [2]Biochemistry, University of VT, Burlington, VT, USA; [3]Neurology, University of VT, Burlington, VT, USA; [4]Medicine, University of VT, Burlington, VT, USA.*

BACKGROUND: Preeclampsia is associated with reduced maternal plasma volume and elevated serum uric acid concentrations. As part of a broader project evaluating pre-pregnancy phenotypes at risk for preeclampsia we sought to determine whether uric acid concentrations are inversely related to maternal plasma volume prior to pregnancy.

METHODS: We examined 50 healthy, non-smoking, nulligravid women during the follicular phase of the menstrual cycle to determine plasma volume. Studies were conducted following 3 days of dietary control and an overnight fast. Plasma volume was estimated employing Evans blue dilution. We measured fasting serum uric acid, blood urea nitrogen (BUN), creatinine (Cr), and 24 hour urinary sodium, volume and creatinine clearance. Data is expressed as mean $\pm$ standard deviation.

RESULTS: Subjects were 29.4 $\pm$ 4.8 years old with a BMI of 22.8 $\pm$ 3.7 kg/m^2. Urinary volumes were 2,439 $\pm$ 1,014 mL and urinary excretion of sodium was 47 $\pm$ 52 mEq/24 hours. The mean concentration of uric acid was 4.1 + 1.0 mg/dL (range 2.1-6.2). We identified no significant association of plasma volume (corrected for BMI) with uric acid concentration (r=0.01, p=0.93). Uric acid concentration were likewise not associated with serum concentration of BUN (r= -0.05, p=0.75), Cr (r= 0.23, p=0.12), 24 hour urinary volume (r= -0.10, p=0.48), 24 hour urinary sodium (r= -0.23, p=0.11) or creatinine clearance (r= -0.18, p=0.26). We did identify a significant association of maternal BMI with serum uric acid concentration (r=0.29, p=0.04).

CONCLUSIONS: Plasma volume corrected for body mass index is not associated with serum uric acid concentrations prior to a first pregnancy. There is evidence that uric acid concentration is associated with maternal BMI. Overall we observed a wide range of values for serum uric acid (3 fold difference). Maternal BMI accounts for approximately 8% of the variance in uric acid concentration. In contrast to prior reports during pregnancy, we found no association of renal function with serum uric acid concentration. Supported by NIH RO-1 HL71944.

766

Hyperuricemia without Proteinuria Is Associated with Adverse Fetal Outcomes in Women with Gestational Hypertension. James M Roberts,[*1] NICHD MFMU Network.[2] *[1]Magee-Womens Research Institute; [2]Bethesda, MD, USA.*

Hypothesis

Gestational hypertension is of greater severity when accompanied by proteinuria (preeclampsia). Proteinuria was chosen historically as one of the first findings accompanying eclampsia (Chesley. Clinical Obstetrics & Gynecology, 27, 81, 1984) rather than for its ability to define severity. In prior work we found that hyperuricemia was at least as effective as proteinuria to identify adverse outcomes as defined by preterm birth and SGA in low risk women with gestational hypertension (Roberts et al. Hypertension 46, 1263, 2005). In the current study we tested the hypothesis that hyperuricemia with gestational hypertension and no proteinuria (HU) would be associated with similar adverse fetal outcomes as hypertension with proteinuria without hyperuricemia (HP) in women at high risk for preeclampsia.

Methods

This is a secondary analysis of data of women with prior preeclampsia from the NICHD MFMU trial of aspirin (ASA) to prevent preeclampsia in high-risk pregnancies. Normal women (NNN) were defined as women with a normal blood pressure, normal uric acid and no proteinuria. ASA did not reduce the risk of preeclampsia and did not affect plasma uric acid concentration in preliminary studies. Thus all women from the trial were included in this analysis. Data analysis was by Fisher's Exact test and log binomial and linear regression.

Results

There were 34 HP, 16 HU women and 131 NNN women. Race, smoking, BMI, maternal age, marital and educational status were not different in HP and HU women compared with NNN women. When compared with NNN, the frequency of preterm birth was greater in HP and in HU, and the frequency of birth weight < 5th centile was greater in HU but not HP women. When HU and HP women were compared, preterm birth (p=0.3) and birth weight < 5th centile (p=0.10) did not differ. When examined as a continuous variable and compared with NNN, the weeks of gestation at delivery for HU were less (36.3 vs. 38.4, p=0.0006) but did not differ for HP (37.8, p=0.18), and HU was less than HP (p = 0.03).

Table 1

	HP (RR [95% CI])	HU (RR [95% CI])
Preterm birth < 37 weeks	2.4 [1.1-5.3]	3.8 [1.7-8.5]
Birth weight < 5th centile	1.3 [0.1-12.0]	8.2 [1.8-37.2]

Conclusion

In women with previous preeclampsia who have gestational hypertension, elevated uric acid even without proteinuria appears to be associated with the adverse outcomes of preterm birth and SGA as commonly as in women with proteinuria without elevated uric acid.

767

2-Hydroxybutanoic Acid; Identified by Metabolomics as a Second Trimester Biomarker for Pre-Eclampsia in Obese Women. Marie Brown,[1] Louise C Kenny,[*2,3] David Broadhurst,[1] Warwick B Dunn,[1] Lucilla Poston,[*4] Shennan H Andrew,[4] Annette Briley,[4] Douglas B Kell,[1] Philip N Baker.[*2] *[1]School of Chemistry, University of Manchester, Manchester, United Kingdom; [2]BUPA Ireland Research Center, Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland; [3]Division of Human Development, University of Manchester, Manchester, United Kingdom; [4]Division of Reproduction and Endocrinology, King's College, London, United Kingdom.*

Objective

Obesity is a known risk factor for preeclampsia. We sought to identify early pregnancy prognostic biomarkers for preeclampsia in a cohort of obese pregnant women.

Methods

Blood samples were obtained from primiparous, obese women (BMI > 30 kg.m^{-2}) taken prior to randomization into the Vitamins in Pregnancy trial between $14^{-0} - 21^{-6}$ weeks gestation. Plasma samples were spiked with internal standard (succinic d_4 acid), derivatised to induce volatility and thermal stability and analysed using GCxGC-TOF-MS. A reference database of metabolite peaks was constructed and for matched metabolites the response ratio was calculated. Univariate methods were employed for data analysis.

Results

For the analysed samples (45 preeclampsia / 39 matched controls) ROC areas were calculated together with the univariate significance of each peak, determined using the Wilcoxon rank sum test. Using thresholds of ROC area > 0.75 and p < 0.01, some individual peaks were identified as being significantly different between cases and controls (outwith Bonferroni correction). One metabolite peak (peak 185), identified as 2-hydroxybutanoic acid was of particular interest (Figure 1. Area under ROC curve vs p value) and given its well-known role in lipid metabolism, may prove a useful quantitative biomarker of preeclampsia risk in the obese patient.

Conclusion

For the first time, we have chemically identified a biomarker for preeclampsia in obese women in the 2nd trimester of pregnancy. These results will be compared with our earlier data [1] obtained from women with established disease.

[1] Kenny LC. et al. (2005) *Metabolomics* ; 1:227-234

Acknowledgements

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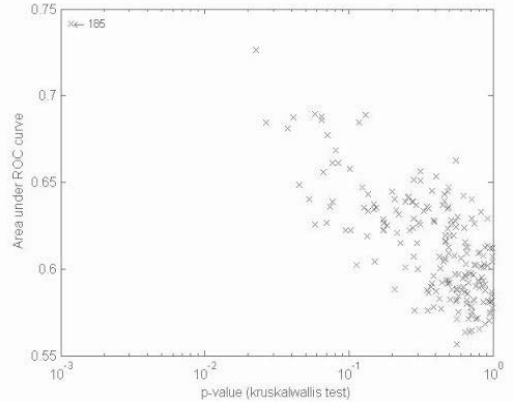

SATURDAY

768

Improved Detection of Metabolic Biomarkers in Pre-Eclampsia. Marie Brown,[1] Louise C Kenny,[*2,3] Warwick B Dunn,[1] David Broadhurst,[1] Douglas B Kell,[1] Philip N Baker.[*3] *[1]School of Chemistry, University of Manchester, Manchester, United Kingdom; [2]BUPA Ireland Research Center, Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland; [3]Division of Human Development, University of Manchester, Manchester, United Kingdom.*

Objective

An earlier study has demonstrated the ability of GC-TOF-MS to detect potential metabolic biomarkers in preeclampsia[1]. A recent analytical technology, comprehensive GCxGC-TOF-MS, was used to analyse both control and disease samples in a preeclampsia study and the results compared to those obtained using GC-TOF-MS to determine whether increased biological information could be obtained.

Methods

20 plasma samples from the GOPEC archive were randomly chosen for each class (disease and control) for analysis. Different samples were chosen for analysis by GCxGC-MS and GC-MS, due to sample volume availability. Samples were spiked with internal standard (0.27mg/ml succinic d_4 acid), derivatised to induce volatility and thermal stability. A reference database of metabolite peaks was constructed and for matched metabolites the response ratio was calculated. ROC areas were calculated together with the significance of each peak (determined using the non-parametric Wilcoxon rank sum (Kruskal-Wallis) test).

Results

Many more peaks were detected using GCxGC-MS than with GC-MS (500 compared to 200). Univariate analyses showed that both techniques detected many peaks (>50) considered to be of biomarker potential, ROC area > 0.75 and statistically significant differences between disease and control ($p<0.01$ using the Kruskal-Wallis test). A Plot of ROC area against probability is shown in Figure 1 (GCxGC-MS) with similar results found using GC-MS, with 10 peaks of biomarker potential being detected using both techniques.

Conclusion

GCxGC-MS detected more peaks than using GC-MS thus increasing the biological information available. A validation study is currently being undertaken where 20 matched disease and control samples from different sample archives are being analysed using both methods.

[1] Kenny LC et al. (2005) *Metabolomics* ; 1:227-234

Acknowledgements

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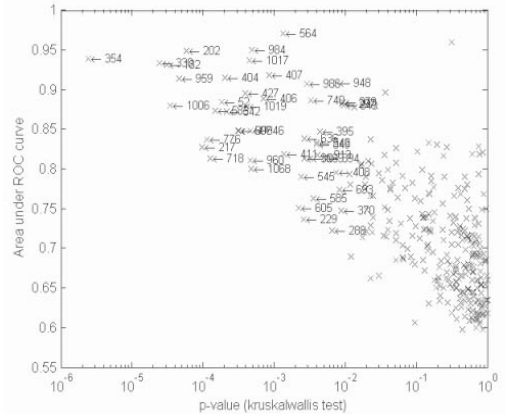

769

Global Gene Analysis Reveals That Siglec-6 Is Increased in Preeclampsia. Virginia D Winn,[1] Ronit Haimov-Kochman,[*1,2] Matthew Gormley,[1] Agnes C Paquet,[3] Ru-Fang Yeh,[3] Nissi Varik,[4] Ajit Varik,[4] Susan J Fisher.[2] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA; [2]Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA, USA; [3]Biostatistics, University of California, San Francisco, San Francisco, CA, USA; [4]Cellular and Molecular Medicine, University of California, San Diego, San Diego, CA, USA.*

Objective: Preeclampsia (PE) complicates ~4-8% of human pregnancies and accounts for significant maternal and neonatal morbidity and mortality. Impaired invasion of placental cytotrophoblasts (CTB) that remodel the uterine vasculature is an established defect in PE. This region of the placenta is known as the basal plate or maternal-fetal interface. Therefore, our goal was to determine the impact of PE on gene expression at a global level at the maternal-fetal interface.

Methods: Informed consent was obtained from all the study participants. Basal plate biopsies were obtained from placentas that were delivered at the conclusion of pregnancies that were complicated by severe PE (n=12; 24 to 36 wks of gestation) or as a consequence of preterm labor with no evidence of infection (n=11; 24 to 36 wks of gestation). The latter samples served as controls. RNA was isolated, processed and hybridized to HG-U133A&B Affymetrix GeneChips. Normalization was performed using Bioconductor AffyPLM software. Statistical significance was set at log-odds ratio B > 0. Q-PCR and immunohistochemistry approaches were used to validate a portion of the differentially regulated genes including Siglec-6.

Results: We identified 71 probesets/55 genes that were differentially expressed in the PE samples as compared to the controls. The differentially expressed genes included molecules previously implicated in PE pathogenesis (e.g., leptin, CRH and sFlt-1) and novel factors such as Siglec-6. Twelve of the differentially expressed genes were validated by Q-PCR or immunohistochemistry analyses. Siglec-6 was expressed by syncyiotrophoblasts as well as invasive CTBs at higher levels in PE as compared to gestational-age matched controls.

Conclusions: Our results reveal a number of novel factors that may play a role in the pathophysiology of PE. In particular, the increased expression of Siglec-6, a transmembrane protein that binds leptin, may serve as a functional receptor for leptin in the human placenta and contribute to the pathophysiology of PE.

770

Identification of Proteomic Biomarkers of Preeclampsia in Amniotic Fluid Using SELDI-TOF Mass Spectroscopy. Joong Shin Park,[1] Kyoung-Jin Oh,[2] Errol Norwitz,[*3] Joong-Soo Han,[2] Hye-Jin Choi,[2] Hyo Sook Seong,[1] Yoon Dan Kang,[1] Chan Wook Park,[1] Byoung Jae Kim,[1] Jong Kwan Jun.[1] *[1]Ob/Gyn, Seoul National University, Seoul, Korea; [2]Biochemistry, Hanyang University, Seoul, Korea; [3]Ob/Gyn, Yale University, New Haven, CT, USA.*

OBJECTIVE: To identify proteomic biomarkers in amniotic fluid (AF) that can distinguish preeclampsia from chronic hypertension and normotensive controls.

METHODS: Under an IRB-approved research protocol, AF was collected from pregnant women with severe preeclampsia (n=13 [sPE]), mild preeclampsia (n=5 [mPE]), chronic hypertension (n=8 [CHTN]), and normotensive controls (n=16 [CTL]) and subjected to proteomic analysis by SELDI-TOF (surface enhanced laser desorption/ionization time-of-flight) mass spectroscopy (Ciphergen Biosystems, Fremont, CA). Proteomic profiles were optimized by varying the ProteinChip binding surfaces and conditions. The chips were read in a Protein Biology System IIC SELDI-TOF mass spectrometer (Ciphergen) using the ProteinChip software v 3.1. High performance liquid chromatography (HPLC), in-gel tryptic digest, and Western blot analysis were used to isolate and identify the biomarkers of interest.

RESULTS: Demographic characteristics (including gestational age) did not differ significantly between the groups. Following proteomic profiling of individual AF samples using the Q10 ProteinChip, two biomarkers of interest were identified: (i) peak X [17399.11 Da] that distinguished women with sPE from mPE, CHTN, and CTL ($p<0.05$); and (ii) peak Y [28023.34 Da] that distinguished women with sPE from mPE and CTL ($p<0.05$). After separation by HPLC, fractions 25 and 33 containing the proteomic biomarkers of interest were subjected to SDS-PAGE gel electrophoresis and in-gel tryptic digestion. The resulting peptides were matched by computer homology to proapolipoprotein A-I (peak Y) and a functionally obscure peptide, SBBI42 (peak X). Western blot analysis confirmed that the AF of women with sPE and mPE had significantly higher proapolipoprotein A-I levels than CTL ($p<0.05$ for both).

CONCLUSION: Proteomic analysis of AF can distinguish women with PE from those with CHTN and normotensive CTL. Proteomic identification techniques matched the discriminatory protein peaks to proapolipoprotein A-I and a functionally obscure peptide, SBBI42. Further studies are ongoing to determine the physiological importance of these proteins in preeclampsia.

771

Preeclampsia Risk: Does Family History Matter? Sindhu K Srinivas,[1] Andrea Goldberg,[1] Mary Sammel,[2] Michal A Elovitz.[*1] *[1]OB/GYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Epidemiology and Biostats, University of Pennsylvania, Philadelphia, PA, USA.*

Background: A positive family history (Fhx) of hypertension and early onset cardiovascular disease (CVD) are strong risk factors for the development of CVD. Studies have suggested that women with a history of preeclampsia (PEC) are at increased risk for developing CVD. We theorize that PEC may, in fact, be

an early presentation of CVD. Therefore, Fhx, specifically multi-generational Fhx, may be associated with the development of PEC. We sought to determine whether a Fhx of CVD in different generations has an impact on PEC risk.
Methods: This study was part of a large case control study, Preeclampsia: Mechanisms and Consequences. Cases are patients prospectively identified with PEC based on maternal criteria. Controls are patients who presented for term delivery. Patients were queried regarding a Fhx of hypertension, diabetes, stroke, coronary artery disease, and myocardial infarction in their grandparents, parents, and siblings. The association of a positive Fhx and PEC risk was evaluated. Individual associations were analyzed using chi square and Fisher's exact tests. The generation-based system quantified the presence of above diseases in the 3 generations with 1,2 and 3 representing the number of generations with a positive history. This scoring system was used to evaluate the association between multi-generational family hx, PEC and severe PEC. Multivariable logistic regression was used to control for confounders.
Results: 248 cases and 232 controls were evaluated. 16.5%, 36.7%, and 46.8% of cases and 85%, 12.7%, and 0.4% of controls had a score of 0, 1, 2+ respectively ($p<0.001$). After controlling for race, maternal age, obesity, hypertension and diabetes, women with a score of 1+ have a 34 times higher odds ([19-60.8], $p<0.001$) of having PEC compared to women with no Fhx (score of 0). A score of 1+ increased the odds of being a mild case by 59.7 ([24.5-145.8], $p<0.001$) and a severe case by 27.2 ([14.5-51], $p<0.001$) when compared to controls.
Conclusion: When applying this unique generation-based Fhx scoring system to our cases and controls, a very strong relationship between family history and PEC is evident. Further investigation is warranted to determine the basis for family history based risk (genetic, environmental, etc) in order to target prevention strategies both for preeclampsia and long-term cardiovascular disease in women.

772

Association of Preeclampsia with the H63D Polymorphism of the Hemochromatosis (HFE) Gene. Carol Lin,[1] Robert E Ferrell,[3] Ashi Daftary,[1] Gail Harger,[4] Marcia J Gallaher,[2] Carl A Hubel.[*1,2] *[1]Dept. of OB/GYN and Reproductive Sciences; [2]Magee-Womens Research Institute, Univ. of Pittsburgh School of Medicine; [3]Dept. of Human Genetics; [4]Dept. of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*
Background: Increased serum iron in women with preeclampsia (PE) could exacerbate free radical formation and endothelial injury. The hemochromatosis (HFE) gene encodes a protein involved in regulation of iron metabolism. At least one function of the HFE protein is downregulation of transferrin-bound iron uptake, thus limiting iron from entering the circulatory pool. Polymorphisms of the HFE gene, H63D and C282Y, appear to compromise the ability of the HFE protein to suppress iron uptake. **Objective:** We asked if the two common HFE polymorphisms, H63D and C282Y, are more prevalent in women with PE than normal pregnancy. **Methods:** Because of the lower frequency of H36D and C282Y polymorphisms in individuals of African descent, and the fewer numbers of these women recruited, analyses were restricted to Caucasian women with PE (n=111 primiparous, 28 multiparous) and normal pregnancy (n=420, all primiparous). PCR amplification was performed using unique sequence flanking primers, and genotyping performed using fluorescence polarization. Allele and genotype frequencies were analyzed using χ^2 contingency tables. **Results:** Allele frequencies adhered to Hardy-Weinberg equilibrium. The frequency of the 63D allele [26% vs. 18%; p=0.006; Odds ratio (OR)1.6 (95% confidence interval 1.13-2.16)] and heterozygous genotype [41% vs. 31%; p=0.014; OR 1.7 (1.10-2.47)] were higher in PE; these differences were unchanged when restricted to primiparous PE women. The frequency of the rare homozygous (DD) genotype was higher in PE overall, but not significantly so [5% vs. 2.6%; p=0.075; OR 2.38 (0.89-6.34)]. However, secondary analysis showed a five-fold higher frequency of DD [14.3% vs.2.6%; p=0.0007; OR 6.8 (1.93-23.9)] among the multiparous women with PE (n=28) compared to controls. In contrast, 282Y allele [4.2% PE vs 6.2% control; p=0.22; OR 0.66 (0.34-1.29)] and genotype frequencies did not differ between preeclamptics and controls. **Conclusion:** Caucasian women who are carriers of the H63D mutation of the HFE gene may be at increased risk of preeclampsia. Funded in part by NIH grants RO1HL64144, MO1RR00056 and PO1HD30367.

773

***STOX1* Gene in Preeclampsia and Intrauterine Growth Restriction.** Anne L Berends,[1] Aida M Bertoli-Avella,[2] Christianne J de Groot,[1,4] Cornelia M van Duijn,[3] Ben A Oostra,[2] Eric A Steegers.[*1] *[1]Obstetrics and Gynecology/Div of Obstetrics and Prenatal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [3]Epidemiology and Biostatistics, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [4]Obstetrics and Gynecology, Medical Center Haaglanden, The Hague, Netherlands.*
Background: *STOX1* gene has been identified as candidate gene for preeclampsia in Dutch females. *STOX1* gene, placentally expressed, is subject to imprinting with preferential transmission of the maternal allele and induces premature throphoblast differentiation resulting in defective throphoblast invasion. Considering the partly common pathogenesis of preeclampsia and intrauterine growth restriction (IUGR) we hypothesized that *STOX1* is involved in the etiology of both disorders.
Objective: To investigate allele frequency differences of *STOX1*- Y153H variation between preeclamptic women and controls and to examine the segregation of *STOX1*-Y153H variation in a separate population of families with pregnancies complicated by preeclampsia or IUGR.
Methods: Allele frequencies were studied in 157 women with preeclampsia and 157 controls in a population based study. Allele transmissions were examined in 50 and 56 families (including parents of cases, spouses and offspring) with preeclampsia and IUGR respectively, originating from a Dutch isolated population. χ^2 statistics were used for analyzing transmission distortion.
Results: *STOX1*- Y153H allele frequencies were not significantly different between preeclamptic women (65%) and controls (64%) in the population based study. Similar frequencies were found in women with preeclampsia and IUGR in the Dutch isolated population. We found no significant evidence for a distortion in transmission of *STOX1*-Y153H variation from mothers with preeclampsia (P=0.21) or IUGR (P=0.17) to offspring.
Conclusions: Our findings neither confirm previous suggestions that *STOX1* is involved in all Dutch preeclamptic patients nor show associations with IUGR. However, involvement of *STOX1* in the pathogenesis of a subset of patients cannot be excluded. The relevance of *STOX1*-Y153H variation in other populations remains to be studied.

774

The Y153H STOX1 Variation Is Not Involved in Severe Early Onset Non-Familial Preeclampsia. Jiska M Jebbink,[1,2] Maarten Buimer,[1] Kees Boer,[1] Joris AM van der Post,[1] Carrie Ris-Stalpers.[2] (SPON: Lucilla Poston). *[1]Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, Netherlands; [2]Laboratory Pediatric Endocrinology, Academic Medical Center, Amsterdam, Netherlands.*
Introduction:
It has been suggested that maternal inheritance of the STOX1 c.457T>C variation, that upon translation results in the p.Tyr153His variation contributes to preeclampsia (PE) within the Dutch population (van Dijk et al, 2005).
Objective:
To determine the relationship between PE and the rs1341667 STOX1 genotype distribution and expression.
Methods:
We analysed the allele frequency of rs1341667 in 36 placentas from normotensive pregnancies and 29 placentas of pathological pregnancies complicated by severe (n=4), early onset (n=19) or severe early onset (n=10) preeclampsia. Severe PE is defined as diastolic blood pressure (BP) >110 mm Hg with proteinuria. Early onset PE is defined by BP >140/90 mm Hg at 2 separate measurements at least 4 hours apart with proteinuria, occurring before 34 weeks' gestational age. 15 of the pathological pregnancies were further complicated by HELLP syndrome. We PCR amplified exon 2 of the STOX1 gene and used HphI RFLP analysis and/or direct sequencing to determine the rs1341667 genotype. RNA was isolated from all placenta tissues heterozygous for the c.457T>C variation. Mono- or biallelic expression was scored after RT-PCR and direct sequencing of the relevant STOX1 fragment.
Results:
There is no significant difference of the T/T, C/C and C/T STOX1 rs1341667 genotype distributions between control and preeclamptic placenta. The C-allele that previously has been implicated in familial preeclampsia is homozygously present in 10 out of the 36 control placenta tissues that show no evidence of any form of gestational hypertensive disorder. The only familial case of PE in our cohort has the T/T genotype. Nine of the 12 control placentas heterozygous for the rs1341667 SNP, express both alleles. Monoallelic expression of the T

allele was observed in 2 control placentas. One placenta shows monoallelic C expression. All 14 placentas from severe preeclamptic pregnancies heterozygous for the rs1341667 variation express both alleles.
Conclusions:
In normal as well as preeclamptic human placenta, both the maternal and paternal STOX1 alleles are expressed. The frequent occurrence of the C allele (61%) in placentas of normotensive pregnancies strongly argues against a role of the Y153H STOX1 variation in the pathogenesis of preeclampsia.

775

Absence of an Association between Tumor Necrosis Factor (TNF) alpha G308A, Interleukin 6 (IL-6) G174C and Interleukin 10 (IL-10) G1082A Polymorphism and Preeclampsia in Caucasian Women. Felix Stonek,[1] Erich Hafner,[1] Martin Metzenbauer,[1] Harald Zeisler,[2] Peter Husslein,[2] Karl Philipp,[1] Walter Tschugguel.*[2] *[1]Danube Hopital Vienna, Department of Obstetrics and Gynaecology, Vienna, Austria; [2]University Clinic of Vienna, Department of Obstetrics and Gynaecology, Vienna, Austria.*
Objective: Preeclampsia (PE) is characterized by hypertension, dyslipidemia and increased systemic inflammatory response. Inflammation markers, such as TNF alpha, IL-6 and IL-10 increased significantly during preeclamptic pregnancy. Whether or not TNF alpha G308A, IL-6 G174C and IL-10 G1082A polymorphisms are involved in the pathogenesis of PE remains to be understood. Recent studies in various genetic populations lead to inconclusive results. The aim of this study was to clarify whether the occurrence of TNF alpha, IL-6 and IL-10 polymorphism is increased in caucasian, mideuropean women who had undergone PE in a previous pregnancy compared to a control group without PE history.
Methods: A retrospective controlled open multicenter study was carried out on 107 women with a history of PE in a singleton pregnancy compared to 107 women with uncomplicated pregnancies. All women were ethnically caucasian. Smears from buccal gingiva cells were analyzed for TNF alpha G308A, IL-6 G174C and IL-10 G1082A polymorphism by hybridisation on microarrays. Statistical analysis was performed by the CHI-Quadrat test.
Results: No difference was found in the number of gene polymorphisms between preeclamptic and control caucasians with heterozygot TNF alpha (29.0 vs 24.3%, p>0.05), IL-6 (46.7 vs 51.4%, p>0.05) or IL-10 (49.5% each) polymorphism, respectively. Moreover, no significance was observed in the number of gene polymorphisms between preeclamptic and control caucasians with heterozygot TNF alpha (1.9 vs 3.7%, p>0.05), IL-6 (17.8 vs 13.1%, p>0.05) and IL-10 (30.8 vs 32.7%, p>0.05) carriers in both groups.
Conclusion: In contrast to other findings, TNF alpha, IL-6 and IL-10 gene polymorphisms fail to be important for the development of PE in our caucasian population. Prospective studies have been initiated to verify these results.

776

Glycodelin Inhibits Progression to S Phase and Cell Proliferation in Human Endometrial Epithelial Cells. Kuniaki Ohta,[1,2] Tetuo Maruyama,[1] Hiroshi Uchida,[1] Masanori Ono,[1] Takashi Nagashima,[1] Toru Arase,[1] Takashi Kajitani,[1] Maki Kagami,[1] Hironori Asada,[1] Mineto Morita,[2] Yasunori Yoshimura.*[1] *[1]Obstetrics & Gynecology, Keio University School of Medicine, Tokyo, Japan; [2]Obstetrics & Gynecology, Toho University School of Medicine, Tokyo, Japan.*
Objectives:
Glycodelin(Gd), a progesterone-induced endometrial glycoprotein, is specifically up-regulated at the initiation of implantaion window and thereafter in endometrial epithelial cells during secretory phase. We have previously reported that histone deacetylase inihibitors induce differentiation and stimulate cell migration in Ishikawa cells, a well-differentiated human endometrial epithelial cell line, through up-regulation of Gd(Uchida, et al, 2005 and in press). In addition to the potential for enhancing cytodifferentiation and cell motility, we here investigate whether glycodelin has an ability to modulate cell cycle progression and cell proliferation.
Methods:
Ishikawa cells were transfected with plasmids encoding enhanced green fluorescent protein (EGFP), EGFP-fused Gd(EGFP-Gd), or EGFP-fused glycodelin lacking exon 4 (EGFP-Gd[del4]). They were then subjected to flow cytometry cell-cycle analysis followed by staining with Hoechst DNA dye. Alternatively, the transfected cells were sorted by flow cytometry according to their EGFP fluorescence intensity and subjected to cell proliferation assay using MTS method. RT-PCR analysis of cyclin-depnendent kinase inhibitors (CDKIs) including p16, p21, and p27 was also performed on total RNA extracted from the transfected cells.
Results:
G1-phase cells were significantly more abundant at 2 days after transfection of EGFP-Gd than that of EGFP alone or EGFP-Gd(del4). Overexpression of EGFP-Gd exhibited a significant reduction in cell number as compared to EGFP or EGFP-Gd(del4) at 3 days after transfection, as determined by the MTS assay. RT-PCR analysis revealed that EGFP-Gd transfection resulted in the up-regulation of p16, p21, and p27 mRNA as compared to EGFP alone.
Conclusions:
These results indicate that Gd inhibits G1/S progression together with up-regulation of CDKIs and thereby reduces cell proliferation. The exon 4-encoding region of glycodelin may have an inhibitory property for cell cycle progression and cell growth. Thus, increased levels of Gd may contribute to endometrial epithelial remodeling during implantation window and also may adversely affect the progression of endometrium-derived diseases including endometriosis and endometrial cancer.

777

The Method of Fertilization (In Vitro vs. In Vivo) Results in Altered Expression of Specific Imprinted and Methylation Genes in Mouse Blastocysts. Gnanaratnam Giritharan, Said Talbi, Francesca Di Sebastiano, Anthony T Dobson, Paolo F Rinaudo. (SPON: Marcelle I Cedars). *Obstetrics, Gynecology & Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.*
Introduction: Increasing evidence suggests that in vitro fertilization and culture may not be completely benign. Cases of Angelman and Beckwith Wiedeman Syndromes, due to aberrant genomic imprinting and abnormalities in growth and development, such as low birth weight, have been described after culture *in vitro*. This study was conducted to explore the effect of the method of fertilization on the expression of imprinted and methylation genes in mouse preimplantation embryos using microarray technology.
Material and methods: CF-1 mice were superovulated with PmSG and hCG. Females were then bred to B6D2F1/J males (IVC group) and zygotes collected the following morning or oocytes were subjected to in vitro fertilization (IVF group). The resulting embryos were cultured to blastocyst stage in Whitten's medium under 5% CO_2 in air. Expanded blastocysts of similar morphology were harvested. Five embryo equivalents of total RNA were amplified, fragmented, labeled and hybridized to Affymetrix mouse 430 2.0 Chip. Each treatment was repeated 4 times. The pair-wise comparison was conducted between IVF and IVC groups to identify specific imprinted and methylation genes with significant differences.
Results: Seventy five imprinted genes and 65 methylation genes were identified on the mouse 430 2.0 Chip. Only 2 imprinted genes (2.6%) and 8 methylation genes (12.3%) show a statistical difference in expression after IVF compared to IVC embryos, as shown in the table below.
Conclusions: The method of fertilization exerts an additional effect on the gene expression level of several imprinted and methylation genes. Overall, there is a down regulation of all the statistically different genes after IVF. Interestingly, Ube3a expression is reduced relative to embryos fertilized in vivo. As Ube3a is associated with Angelman Syndrome, this finding needs to be further investigated.

Expression levels of statistically significant imprinting and methylation genes in IVF vs. IVC blastocysts

Methylation Genes	**Expression**
2410012M04Rik	0.79
Hdac10	0.73
Hdac8	0.89
Mecp2	0.69
Nnmt	0.86
Pnmt	0.71
Suv39h1	0.86
Tyms	0.63
Imprinted Genes	**Expression**
Slc22a3	0.73
Ube3a	0.81

778

Estrogen Stimulates Relaxin Receptor LGR7 mRNA in an In Vitro Model of Human Term Pregnancy Cervix. Priya B Maseelall, Gerson Weiss,* Andrea Wojtczuk, Laura T Goldsmith.* *Obstetrics, Gynecology, and Women's Health, New Jersey Medical School, UMDNJ, Newark, NJ, USA.*
Objective: Relaxin is an important remodeller of female reproductive tract connective tissue. Some actions of relaxin appear to require exposure to estrogen. The precise role of estrogen in the cellular response to relaxin is not defined in any relaxin target tissue. We tested the hypothesis that relaxin action in the cervix is modulated by estrogen by determining expression of the LGR7

relaxin receptor, a G-protein coupled, leucine rich repeat protein, and whether estrogen regulates it in a well established, relaxin responsive, in vitro model of human term pregnancy cervix, lower uterine segment fibroblasts (LUSF).

Materials and Methods:

LUSF were incubated in the presence and absence of estradiol (1μM). Total cellular RNA was isolated and reverse transcribed into cDNAs. Real-time PCR, using primers verified for human LGR7, was used to amplify specific sequences from the cDNAs. Rhesus monkey skeletal muscle and myometrium were used as negative and positive tissue controls. Reactions used SYBR-Green fluorescent detection of double stranded DNAs. Absolute quantification determined amounts of LGR7 receptor mRNA. Standard curves used purified PCR product of known concentration, generated from RNA isolated from human endometrial glandular epithelial cells. PCR products from LUSF experiments were assessed for size by agarose gel electrophoresis and specificity by melt curve analysis and nucleotide sequencing.

Results:

LGR7 mRNA was detected in LUSF. The expected amplicon size of 192 base pairs was detected and a single peak at 83.3°C was seen on melt analysis in PCR products generated from RNA of control and estrogen treated cells. Nucleotide sequencing revealed 100% homology with the reported human LGR7 sequence (NCBI accession # NM_021634).

LGR7 mRNA levels in LUSF were significantly increased by estradiol to mean levels of 146% ± 6.5 (M ± SE, n=2 cell experiments each performed using multiple RT-PCR reactions) above those of control, untreated cells ($p = 0.04$).

Conclusions:

These data are the first demonstration of estrogen regulation of relaxin receptor expression. That estrogen positively regulates relaxin receptor expression in LUSF, suggests estrogen amplification of relaxin action in the cervix.

Supported by NIH grant HD22338.

779

Endometrial Database (EDB), a New Online Tool for Endometrial Research. Jose A Horcajadas,[1] Julio J Merelo,[2] Francisco Dominguez,[1] Sonia Molina,[2] Antonio Pellicer,[*1] Carlos Simon.[*1,3] *[1]Fundacion IVI-Instituto Universitario IVI-University of Valencia, Valencia, Spain; [2]Universidad de Jaen, Jaen, Spain; [3]Centro de Investigacion Principe Felipe-University of Valencia CIPF/UVEG, Valencia, Spain.*

Objectives: The endometrium is a highly hormonally regulated organ. An emerging body of new information for genes that control hormonal development in human endometrium is becoming available. Our aim was to create an Endometrial Database (EDB) to include and organize all the information related to genes involved in endometrial function.

M&M: the EDB is located at the URL http://www.endometrialdatabase.com/. The EDB is implemented as a relational database using a MySQL server running on a dedicated Linux Kubuntu 6.10 system. It consists of several tables with the information about the gene expression data that has been published in each category. If available, functional information is included. Links to the corresponding biomedical publications and nucleotide and protein database are also present. Initially, the website was developed using HTML KIT. Active Server Pages code as the interface to the database. The Active Server Pages were later replaced by DTML and SQL Methods embedded in JOOMLA, a Content Management System (CMS) and Web Application Framework, installed on server with LAMP Tecnology.

Results: We have initiated an Internet database project for the benefit of researchers working in endometrium named Endometrial Database (EDB). This online tool contains data from genes expressed in the endometrium from different species. In the human endometrium, these data are classified into several categories: natural cycles, stimulated cycles, contraception, endometriosis and endometrial cancer. Other categories include animal and in vitro models. Furthermore, it provides links to other online information about nucleotide and amino acid sequences and related publications. Researchers can know how one gene is regulated in the different situations only by introducing the gene name. Scientists can participate by submitting new information to the database and updating existing data.

Conclusions: From Fundacion IVI-Univ. of Valencia, we have created the EDB to provide a unified online gateway to store, search, review, and update information about genes and biological processes expressed in the endometrium. It will be adapted to reflect the current state of the art about genes and functions expressed in the human endometrium.

Supported by Organon-Spain.

780

Expression of Leucine-Rich Repeat G-Protein-Coupled Relaxin Receptors in Rhesus Monkey Reproductive Tissues. Dorette Noorhasan, Priya Maseelall, Jeff Gardner, Robert Donnelly, Andrea Wojtczuk, Gerson Weiss,[*] Laura T Goldsmith.[*] *Obstetrics, Gynecology, & Women's Health, New Jersey Medical School, Newark, NJ, USA.*

Objectives: Our objective is to enhance the understanding of relaxin actions in primate species. To this end, we have completed assessment of reproductive tissue distribution of relaxin receptors LGR7 and LGR8, and determined the role of relaxin in receptor expression.

Materials and Methods: Female monkeys were ovariectomized, steroid primed, and randomized to H2 relaxin (n = 4) or vehicle treatment (n = 4) to simulate early pregnancy. After twenty-one days, tissues were removed, RNA extracted, and subjected to real-time RT-PCR using primers which amplify specific nucleotide sequences in monkey LGR7 and β-actin, and human LGR8. Melt curve analysis, agarose gel electrophoresis, and nucleotide sequence analysis were used to confirm amplicon composition. The comparative cycle threshold method ($2^{-\Delta\Delta Ct}$) determined relative expression of LGR7 and LGR8 mRNA in each tissue type, in control and relaxin-treated monkeys.

Results: LGR8 mRNA is detectable in myometrium, cervix, endometrium, and vagina, but not in mammary gland. Analyses of 199-nucleotide LGR8 amplicons from all tissues revealed one nucleotide difference from the reported human LGR8 nucleotide sequence, corresponding to no variation of the monkey amino acid sequence from the human. Relaxin does not positively regulate expression of LGR8 mRNA in monkey reproductive tissues as indicated by mRNA fold changes in relaxin treated animals of 0.89 in myometrium, 2.73 in cervix, 0.43 in endometrium, and 1.13 in vagina.

We have previously shown LGR7 mRNA detection and lack of regulation by relaxin in Rhesus monkey myometrium, cervix, and endometrium. Completion of our tissue distribution analysis revealed LGR7 mRNA expression in vagina and mammary gland which was not positively regulated by relaxin as indicated by LGR7 mRNA fold changes of 1.12 in vagina and 0.33 in mammary gland.

Conclusions: This is the first demonstration of LGR8 receptor expression in nonhuman primates. The widespread tissue distribution of both receptors suggests extensive effects of relaxin in primate reproductive tissues.

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781

Role of Superoxide and Nitric Oxide in the Development of Postoperative Adhesions. Ghassan M Saed, Zhong L Jiang, Michael P Diamond,[*] Husam M Abu-Soud. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*

Introduction: Adhesion fibroblasts are characterized by lower levels of nitric oxide (NO) as compared to normal peritoneal fibroblasts; although there was no difference in the expression levels of iNOS (inducible Nitric Oxide Synthase), the enzyme that produces NO. Myeloperoxidase (MPO) is known to consume NO and generate reactive oxygen and nitrogen species, which may play a role in the pathogenesis of postoperative adhesions. The hypothesis to be tested is that superoxide down regulates MPO and iNOS expression through a mechanism which leads to decreased bioavailability of NO.

Objective: To test the effect of xantheine/xantheine oxidase, a superoxide generating system, on the expression of iNOS and MPO in normal and adhesion fibroblasts.

Methods: Primary cultures of human normal peritoneal and adhesion fibroblasts were cultured under normoxic conditions using standard techniques. iNOS and MPO mRNA and protein levels were measured by the real time RT-PCR and immunoflorucence. Nitrite/nitrate levels were measured by Griess assay. Cells were treated for 24 hrs with xantheine (100 $_{\mu}$M)-xantheine oxidase (5 $_{\mu}$U) and SNAP (S-nitroso acetyl penicillamine), an NO donor (100 $_{\mu}$M).

Results: Adhesion fibroblasts have a significantly lower MPO mRNA and protein levels as compared to normal fibroblasts. There were no differences in iNOS mRNA and protein levels in both cell lines. Treatment with hypoxia resulted in a significant increase in iNOS, but, decreased MPO mRNA and protein levels. However, treatment with xantheine/xantheine oxidase resulted in a significant decrease in NO levels and in mRNA and protein levels for both iNOS and MPO. Opposite effects have been observed when cells were treated with SNAP.

Conclusions: Our data clearly indicate that NO is important in modulating iNOS and MPO gene expression in normal and adhesion fibroblasts. Limiting the bioavailability of superoxide (i.e., less exposure to hypoxia) may be a potential target for intervention to protect against the development of the adhesion phenotype.

782

Side Population Cell Expression in a Uterine Endometrium-Injury Mouse. Shinji Hyodo, Keiichi Matsubara, Masaharu Ito. (SPON: Ronald R Magness). *Obstetrics and Gynecology, Ehime University School of Medicine, Toon, Ehime, Japan.*

Objectives: Endometrial implantation disorder is responsible for limiting the success rate of in vitro fertilization-embryo transfer (IVF-ET). Cause of the disorder is classified into uterine endometrium insufficiency and abnormal embryo. Two-step embryo transfer is performed for endometrium insufficiency; however, the success rate of IVF-ET is not yet acceptable. In this study, we focused on Side population cells (SP cells), which is an attractive research field of regenerative medicine.

Material and methods: We made a uterine endometrium injury model mouse using lipopolysaccharide (LPS) injection into the peritoneal cavity. To investigate the time course of the regeneration of uterine endometrium, HE staining was performed on uterine specimens obtained from these mice. Next, we measured the number of SP cells in the fraction of epithelium and interstitium of the uterine endometrium using flow cytometry. Furthermore, the endometrium was immunostained with anti- Breast cancer resistance protein (BCRP) antibody and the distribution of SP cells were analyzed.

Results: Injury of uterine endometrium was observed 6 hours after LPS injection and recovered 16 hours later. SP cells were significantly increased, showing a peak in stromal cell 6 hours after LPS injection and the number in the epithelium was increased 12 hours later. This phenomenon was also demonstrated by immunohistochemistry with anti-BCRP antibody.

Conclusions: SP cell might play an important role in the regeneration of uterine endometrium.

783

Detailed Ultrasound Real-Time Velocity Profiles Using a Novel Global Acquisition & Signal Processing (G.A.S.P.) Software for the Multigate Spectral Doppler Analysis: Uterine and Fetal Hemodynamic in Very Early Pregnancy. Gabriele Urban,[1] Michael J Paidas,[2] Stefano Ricci,[3] Fabio Sanguineti,[2] Piero Tortoli,[3] Patrizia Vergani,[1] Charles J Lockwood,[*2] Pasquale Patrizio.[2] *[1]Obstetrics and Gynecology, University of Milan Bicocca, Monza, Italy; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [3]Engeneering and Telecomunications, University of Florence, Florence, Italy.*

Objective: To study materno-fetal hemodynamic in very early pregnancy. In this paper we propose a new US method, named Multigate Spectral Doppler Analysis (MSDA), that overcomes the limitation related to the use of a single sample volume. In this method, 128 small sample volumes are aligned along an US scan line that intercepts the vessel. The Doppler data from each sample volume can be independently analysed to produce a high resolution flow profile.

Methods: The Multigate Spectral Doppler Analysis (MSDA) system employed in this study consisted of commercial ultrasound machine (Aloka SSD1400) connected to personal computer (PC) where a proprietary electronic board was plugged and where the G.A.S.P software ran. Imaging was performed within standard safety guidelines. We scanned left and right uterine arteries and fetal aorta in 30 pregnant women at 6 week and at 9 week of gestation after in-vitro fertilization. We evaluate velocities profiles, wall distension rate and shear rate calculating from seven consecutives cycles.

Results: We were successfully able to generate a velocity profiles from all the vessels interrogated maternal and fetal in real-time. Qualitative description of flow is consistent with the expected laminar flow pattern of those vessels. Comparables conventional Doppler spectrums corresponds to different velocity profiles, WSR and WDR.

Conclusions: We found a substantial difference between conventional Doppler results and velocity profile from MGSD. Qualitative description of flow show the complete distribution across the lumen instead of cross-sectional mean which preserve his profile during consecutive cycles. Maximal velocities in the center of the vessel are higher compared to PSV whether mean velocities are lower, highlighting the fact that same PSV could masks different kind of pattern flow, like turbulent and pseudo-laminar not always cleared by the presence of notch. Physiologic notch don't show in any of the cases studied a reverse flow.

784

In Vitro Effects of Phthalic Acid Mono-n-butyl Ester on Early Mouse Embryo Development. Nam D Tran, Gnanaratnam Gnanaratnam Giritharan, Victor Y Fujimoto, Peter C Klatsky, Paolo Rinaudo. (SPON: Linda C Giudice). *Department of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: The diesters of 1,2-benzenedicarboxylic acid, commonly known as phthalates, are synthetic chemicals often used as platicizers in the manufacture of flexible vinyl. Recently, phthalate esters have been shown to disrupt implantation and pregnancy maintenance in mated rats. These studies investigated the effects of mono-n-butyl ester phthalate, (MBP), on early mouse embryo development.

Methods: Six-week-old CF1 female mice were hyper stimulated with PSG and mated to C57/Bl6 males after hCG injection. Zygotes were harvested from successfully mated females 20 hours after hCG injection and exposed to different concentrations of MBP in vitro immediately after collection or at the 2-cell stage. Embryo development was then evaluated daily.

Results: MBP exposure significantly decreased cleavage rate of zygotes at 24 hours in a dose-dependent manner. Similarly, MBP also negatively effected the development of morulas and blastocysts, at 72 hours and 96 hours, respectively. Toxic concentration of MBP ranged between 1mg/ml to 10mg/ml. Viable zygotes were not observed after 24 hours of MBP exposure at concentration of 10mg/ml. MBP exposure at concentration of 0.1mg/ml did not seem to disrupt cleavage, or morula and blastocyst formations. As expected, zygotes were able to tolerate higher concentration of MBP with less disruption to embryo development when MBP exposure was delayed for 24 hours. This was evidenced by the development of morulas and blastocysts at concentrations that were toxic when zygotes were immediately exposed to MBP without delay.

Conclusion: These results demonstrated that MBP significantly interrupted early mouse embryo development in a dose-dependent fashion. Additionally, these data also showed that early exposure to MBP was more detrimental to later exposure.

785

Quantification of Sperm hCG Receptor (hCG-R) mRNA and Its Potential Effects on Sperm Function. Rene B Allen, Aimin Li, Frank Z Stanczyk,[*] Richard J Paulson,[*] Rebecca Z Sokol. *Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, CA, USA.*

Introduction:

Recent studies suggest a role for hCG treatment for men with poor semen quality prior to TESE and of semen samples in vitro prior to ART, suggesting a role for sperm hCG-R. These hCG-R have been demonstrated with immunohistochemistry. However, PCR is superior as it permits quantification of mRNA, and thus, an evaluation of the relationship between sperm parameters and the amount of hCG-R mRNA.

Objective:

1) To quantify sperm hCG-R mRNA using PCR.

2) To investigate the relationship between sperm hCG-R mRNA content and markers of spermatogenesis and sperm function.

Methods:

Ten men, aged 26 to 46 years, provided semen samples. Samples were analyzed for volume, concentration, motility, and morphology.

cDNA was generated by reverse transcription of 2 µg of total RNA, after TRIzol extraction from sperm. Endometrium cDNA was a control since it expresses hCG-R mRNA. HCG-R mRNA was amplified in duplicate with a primer pair designed using LightCycler Probe Design Software. The amplicons, amplified for 50 and 30 cycles, were run on ethidium bromide (EB) stained agarose gels.

A relative quantification analysis of hCG-R mRNA was carried out with the LightCycler software, v4. The ratio of hCG-R DNA sequence to a reference DNA sequence, GAPDH from sperm, was determined and compared to the ratio of the same sequences in samples from endometrium, which served as a control "Calibrator" (1x sample). The results are expressed as a normalized ratio.

Results:

Median semen analysis parameters (ranges) included a volume of 2.4 ml (0.5-3.0 ml), a concentration of 80.5 million/ml (26-173.5 million/ml), a motility of 62.5% (50-75%), and a morphology of 11.5% (5-17%). The median normalized ratio of hCG-R DNA to GAPDH DNA was 25.3 (24.2-30.9).

The EB stained agarose gel revealed a distinct band for each sample at 299 base pairs for hCG-R, the size of our primer pair. Receptor expression varied significantly between samples when amplified with fewer cycles. Linear regression analysis revealed a statistically significant association between hCG-R and sperm concentration ($r2=0.63$, $p=0.01$).

Conclusion:
1) We demonstrated the presence of hCG-R mRNA in spermatozoa; to our knowledge this is the first report demonstrating sperm hCG-R mRNA in ethidium bromide stained agarose gels.
2) The positive correlation between hCG-R mRNA content and sperm concentration suggests a role for seminal plasma hCG in spermatogenesis.

786

Free Radicals and Oocyte Aging. PT Goud, AP Goud, Michael P Diamond,* Bernard Gonik,* HM Abu-Soud. *Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*
The free radical, superoxide ($O_2^{\bullet-}$) is a major contributor to oxidative stress. $O_2^{\bullet-}$ and its downstream molecules, not only damage cell organelles and DNA, but also affect the synthesis/activity of oocyte factors crucial for fertilization and embryo development. Increased $O_2^{\bullet-}$ reacts with and consumes nitric oxide (NO), which is critical to prevent oocyte aging and maintain spindle integrity. Moreover, its relatively stable product, hydrogen peroxide (H_2O_2) can alter oocyte Ca^{2+} release akin to postovulatory aging. However, direct effect of $O_2^{\bullet-}$ on the oocyte is not well studied. We therefore, study the effects of $O_2^{\bullet-}$, H_2O_2, and hypochlorous acid (HOCl) on the oocyte. **Methods:** Freshly ovulated mouse oocytes were exposed either to $O_2^{\bullet-}$ [hypoxanthine/xanthine oxidase system generating 1.2 (n=42) and 2.5 μM $O_2^{\bullet-}$/min (n=45)], H_2O_2 (20 or 100 μM, n=60), hypochlorous acid (HOCl, 1, 10 and 100 μM, n=50), and their sibling oocytes were fixed immediately or cultured under physiological conditions (n=96). Oocytes were then assessed for the aging phenomena by judging the zona pellucida dissolution time (ZPDT) under light microscopy and ooplasm microtubule dynamics (OMD) and cortical granule (CG) status using fluorescence immunocytochemistry and confocal microscopy. **Results:** Although ZPDT (seconds, Mean±SD) increased in relatively old (36.1±6.2) compared to young untreated oocytes (17.7±4.1, $P<0.0001$), exposure to superoxide further increased ZPDT at 1.2 and 2.5 μM ($P<0.0001$). Similarly, $O_2^{\bullet-}$ exposure resulted in significantly more oocytes with increased OMD and major CG loss (1.2 μM : 71.4 and 64.3%; 2.5 μM: 88.9 and 84.4%) and fewer oocytes with minimal OMD and intact CG (1.2 μM : 0 and 7.1%; 2.5 μM: 0 and 0 respectively) compared to untreated controls (increased OMD and major CG loss: 16.0 and 8%; minimal OMD and intact CG: 20.0 and 32% respectively, $P<0.0001$). Interestingly, young oocytes exposed to 20 μM H_2O_2 resisted aging phenomena, while 100 μM enhanced aging. Nonetheless, relatively old oocytes showed sensitivity to both concentrations *(*$P<0.05$). Enhanced ZPDT, OMD and CG loss were also seen in oocytes exposed to HOCl at 1 and 10 M, while higher concentrations (100 μM) compromised oocyte viability. **Conclusions:** 1) $O_2^{\bullet-}$, H_2O_2 and HOCl augment aging phenomena in oocytes. 2) Young oocytes are resistant to H_2O_2-induced aging relative to old oocytes indicating improved antioxidant machinery in young versus old oocytes. 3) $O_2^{\bullet-}$ and downstream products play an active role in regulating oocyte aging.

787

Contraceptive Activity of Low Dose GnRH II Analog. Theresa M Siler-Khodr,*[1] Fu-Quig Yu,[2] Peng Wei,[2] Shi-Xin Tao,[2] Susan Coulhart,[1] Shari Mactyszczyk,[1] Lui Yi-Xun.[2] *[1]Contraceptive Research, Center for Investigation of Cell Regulation and Replication, San Antonio, TX, USA; [2]The State Key Laboratory of Reproductive Biology, Institute of Zoology, Beijing, China.*
Objective: Two GnRH isoforms are produced in primates, such as monkeys, baboons and humans. In these species, GnRH II (chicken II GnRH) is produced at multiple sites, including the placenta, ovary, uterus and the brain. Its LH releasing activity is limited as compared to the GnRH I (mammalian GnRH). However, we and others have demonstrated that GnRH II has direct actions at extrapituitary sites. We have designed a GnRH II analog with potent contraceptive activity. This contraceptive activity exists in the presence of normal luteal progesterone production, leading us to propose that another mechanism of action affects this activity. In these studies we defined the contraceptive activity of GnRH II in relation to ovulation. **Methods:** Cynomolgus monkeys (n=26) were mated with males for two days between –2 to +2 days of ovulation, and then treated with saline (n=14) or GnRH II analog via osmotic minipumps (32 ug / day for six days, n=12). Blood was sampled prior to mating, after mating just prior to analog or vehicle treatment, and biweekly thereafter. The time of ovulation was established by the E2 and LH peak and subsequent increase in progesterone. Delivery of the analog was confirmed by a specific RIA for the analog. Implantation rate was determined by mCG and pregnancy rate by term deliveries. **Results:** Luteinization was not inhibited by this dose of GnRH II analog, nor was cycle length disturbed. Implantation rate was 43% in saline treated animal, and term pregnancy rate in the controls was 36%. GnRH II analog treated animals had two implantations of 12 analog treated animals (17%), and only one going to term (8%). Both implantations occurred in animals that were treated +2 days post ovulation. Contraceptive efficacy was 100% in animals beginning treatment prior to or on day +1 post ovulation. **Conclusions:** These findings confirm that low-dose GnRH II analog effects contraceptive activity without affecting luteal steroids. The post-coital contraceptive activity was 100% even two days post-coital provided the GnRH II analog treatment was prior to 2 days post ovulation. This timing suggests contraceptive activity for low dose post-coital GnRH II analog acts during fertilization, possibly via a sperm-egg interaction.

788

Identification of Human Embryonic Poly(A)-Binding Protein (ePAB). Samuel A Pauli, Maria D Lalioti, Ozlem Guzeloglu-Kayisli, Denny Sakkas, Emre Utku Seli.* *Department of Obstetrics & Gynecology, Yale University School of Medicine, New Haven, CT, USA.*
Hypothesis: Regulation of gene expression in the mature oocyte and the early embryo occurs primarily by translational activation of maternally derived mRNAs by extension of their poly(A) tails. This process called cytoplasmic polyadenylation is mediated by an embryonic poly(A) binding protein (ePAB) and lasts until activation of zygotic transcription. We have previously identified and cloned the mouse ortholog of Xenopus ePAB, and showed that it is expressed in oocytes and in embryos up to the 4-cell stage. In this study we hypothesized that similar mechanisms may take place in humans and sought to identify and characterize human ePAB.
Methods: Nucleotide and protein sequence databases were searched using standard nucleotide-nucleotide BLAST (blastn) and protein query vs. translated database BLAST (tblastn) using the National Center for Biotechnology Information BLAST server. Pairwise and multiple alignments of the human, mouse, and Xenopus ePAB genes and proteins were performed using the MegAlign program (DNASTAR Inc., Madison, WI). The prediction and assignment of the protein structures were performed using Pfam (http://pfam.wustl.edu). Oocytes (prophase I), blastocysts, and twelve different tissues, including testes and ovaries were tested by reverse transcription polymerase chain reaction (PCR) and real-time PCR for the expression of ePAB and somatic cell cytoplasmic PABP (PABPC1) mRNA. G3PDH amplification provided a positive control and allowed semi-quantitative analysis.
Results: Human ePAB is a 625 aa protein with 71% identity and 79% similarity to mouse ePAB and contains 4 RNA recognition motifs and a PABP domain. Human ePAB mRNA was detected in ovarian tissue and to a lesser extent in testes and several somatic tissues including kidney, liver, lung, and muscle. Similar to the mouse ortholog, human ePAB mRNA was expressed in oocytes. As expected, PABPC1 mRNA was ubiquitously present in all tissues as well as blastocyst stage embryos.
Conclusions: In this study we report the identification of human ePAB. Human ePAB shows high identity to its mouse and Xenopus orthologs and its expression pattern mimics that observed in other vertebrates although it is less restricted to gonads. The detection of an ePAB in human oocytes suggests that the unique translational regulatory pathways that control gene expression in oocytes and early embryos may be common between model organisms and humans.

789

Improved Fertilization after ATP Treatment of Sperm. Scott E Edwards,[1] Mariano Buffone,[1] Marco Rossato,[2] Stuart B Moss,[1] Carmen J Williams.*[1] *[1]CRRWH, Univ. PA, Philadelphia, PA, USA; [2]Dept. Med. Surg. Sci, Univ. Padova, Padova, Italy.*
Background: Sperm capacitation is regulated partly by molecules found in the female reproductive tract. Extracellular ATP is present in the female reproductive tract and a clinical study (Rossato et al., Hum Reprod 1999, 14:694) showed improved in vitro fertilization (IVF) when sperm were treated with ATP.
Objective: To quantify the effects of ATP on markers of mouse and human sperm function, including motility, acrosomal exocytosis (AE), protein tyrosine phosphorylation, and IVF success.
Methods: Human AE was analyzed using the PSA-FITC assay. Mouse sperm AE was determined by analyzing sperm from transgenic mice expressing acrosin-GFP. Protein tyrosine phosphorylation was determined by immunoblotting. Sperm motility parameters were assessed using computer aided sperm analysis. Mouse IVF was performed such that the control fertilization was between 20-50%. Fertilization was defined by the presence of two pronuclei and subsequent cleavage.

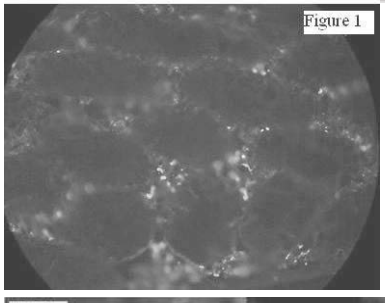
Results: For mouse sperm, ATP improved IVF when using either cumulus cell-free (control 16.1%, ATP 63.3%; p<0.0001) or cumulus cell-intact eggs (control 52.9%, ATP 73.4%; p<0.001). ATP had no effect on mouse sperm AE and did not increase protein tyrosine phosphorylation. However, ATP altered sperm motility parameters including progressive velocity (control 112.4 ± 9.9, ATP 154.8 ± 9.1 μm/sec; p=0.01) and linearity (control 38.4 ±2.1, ATP 46.6 ±2.3; p=0.03). A nonhydrolyzable ATP analog, AMP-PCP, similarly stimulated mouse sperm motility. ATP treatment of sperm from fertile men did not affect AE or protein tyrosine phosphorylation, but it did alter sperm motility parameters by increasing curvilinear velocity (control 122.5 ±3.5, ATP 144.8 ±7.6 μm/s; p=0.02) and hyperactivation (control 7.8 ±1.9%, ATP 21.3±5.3%; p=0.03). After ATP treatment, sperm from asthenozoospermic men demonstrated increased progressive velocity (control 23.8, ATP 28.8 μm/sec; p=0.002) and linearity (control 29.6, ATP 37.7; p<0.001).
Conclusions: Mouse sperm treated with extracellular ATP manifest enhanced fertilization capacity in vitro. This improvement may be due to changes in the sperm motility characteristics. ATP exerts similar effects on human sperm, which could explain its beneficial effects on human IVF success. These results suggest that ATP could constitute a new therapeutic modality in the treatment of male infertility.
Grant Support: Duska Therapeutics.

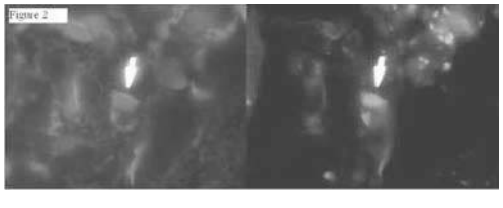
790

A Potential Role for Hematopoetic Stem Cells in the Regeneration of Spermatogenesis. Ahmad O Hammoud,[1] Mark Gibson,[*1] C Matthew Peterson,[1] Harry Hatasaka,[*1] Jerry Spangrude.[2] *[1]Reproductive Endocrinology and Infertility, University of Utah, Salt Lake City, UT, USA; [2]Hematology, University of Utah, Salt Lake City, UT, USA.*
Objective: The purpose of this study is to investigate the ability of hematopoietic stem cells (HSC) to engraft into testicular tissue and to express a germ cell phenotype.
Material and methods: Donor mice engineered to broadly express the molecular tag EGFP served as a source of bone marrow cells, which injected retro-orbitally into radiation-sterilized wild type mice. Recipient mice were sacrificed 2 and 3 months after transplantation. Testicular tissue was fixed with formalin then cryo-preserved. Donor cells were identified by expression of EGFP. Evidence expression of features of spermatogenic potential in EGFP-positive cells was proven through co- localization of the germ tissue-specific antigen VASA (Noce et al, 2001) using a polyclonal chicken anti-VASA primary antibody followed by anti-chicken antibody conjugated to Alexafluor 594.
Results: We started with a group of 4 wild type mice. Two were sacrificed at 2 months and the rest were sacrificed 3 months after the procedure. Evidence of hematopoietic cell engraftment in the testicular tissue was demonstrated by the existence of EGFP-positive cells in the testicular tissue (Figure 1). These cells derived from hematopoietic cells were found at 2 and 3 months after transplantation.
VASA staining of the transferred cells localized to the testis showed that a number of cells expressed VASA antigen (Figure 2) and (Table 1).
Conclusion: HSC are capable of engrafting into testicular tissue after intravenous transplantation. These cells can express VASA, a germ cell specific antigen.

Table 1

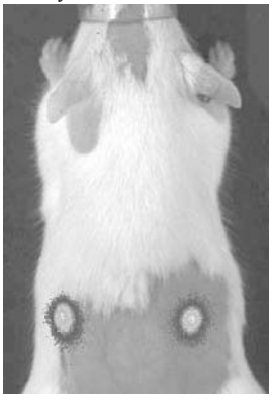
Period after transplantation	EGFP positive cells in 20 power fields	EGFP positive cells expressing VASA
2 months	250	3
3 months	473	8





791

Identification of Mouse GnRH Gene Promoter Sequences Critical for Ovarian Expression in Live Mice. Rebecca F Lara, Keeley L Mui, Amisra A Nikrodhanond, Helen H Kim.[*] *Obstetrics & Gynecology, University of Chicago, Chicago, IL, USA.*
Objective: Gonadotropin-releasing hormone (GnRH) is produced in the ovary and modulates ovarian function. To elucidate mechanisms regulating ovarian GnRH, our objective was to isolate the DNA sequences critical for ovarian GnRH expression in a transgenic mouse model, using whole-body imaging.
Methods: We previously generated transgenic mice with various fragments (-249, -2078, -3446 bp) of the mouse GnRH gene promoter fused to the luciferase reporter gene (LUC). In these mice, the anatomic pattern of luciferase activity reflects GnRH gene promoter activity. For whole-body imaging, luciferin was administered. GnRH promoter activity was visualized in live GnRH-LUC mice as bioluminescence (photons/sec). For each transgenic line, 3-5 female mice were examined. After whole-body imaging, luciferase activity was measured as relative light units (RLU) in tissue homogenates. Luciferase activity obtained by these methods was compared.
Results: Luciferase activity was detected only in the brain and ovary. Overall, luciferase activity detected by whole-body imaging correlated with values from tissue homogenates. The lowest level of ovarian luciferase activity (18,824 ± 3,777 photons/sec and 816 ± 53 RLU) was detected in the -3446 LUC mice. Luciferase activity in the -2078 LUC mice was the highest (305,779 ± 68,940 photons/sec and 21,610 ± 2,710 RLU). Ovarian luciferase activity in the -249 LUC mice was 61,022 ± 8,955 photons/sec and 1,901± 224 RLU. The **figure** shows ovarian bioluminescence in a GnRH-LUC mouse.
Conclusions: Whole body imaging demonstrates that promoter elements, contained within the proximal -249 bp of the mouse GnRH gene promoter, mediate GnRH gene expression in the ovary. In contrast, the distal portion of the GnRH promoter, between -3446 and -2078 bps, appears to mediate ovarian repression of GnRH expression since deletion of this region unmasks ovarian expression. Our studies demonstrate that whole-body imaging can isolate the DNA sequences critical for ovarian GnRH expression in GnRH-LUC transgenic mice and is a powerful tool that permits assessment of promoter activity in live animals.



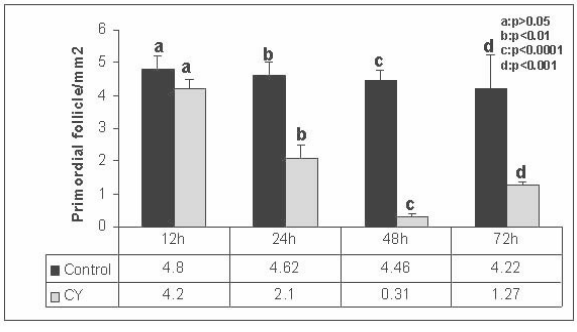
792

Proliferation of Theca-Interstitial Cells Is Sensitive to Inhibition of Glycosylation. Antoni J Duleba,[*1] Piotr C Piotrowski,[1,2] Jakub Kwintkiewicz,[1] Izabela J Rzepczynska.[3] *[1]OB/GYN, Yale University, New Haven, CT, USA; [2]Polish Academy of Sciences, Warsaw, Poland; [3]Gyn/Ob, Poznan University of Medical Sciences, Poznan, Poland.*
Introduction: Recently, in a randomized clinical trial, we found that simvastatin decreases testosterone level in women with polycystic ovary syndrome (PCOS). This effect correlates well with our previous observation that statins inhibit proliferation of ovarian theca-interstitial (TI) cells - a primary source of testosterone in PCOS. The effects of statins on TI cell proliferation are independent of cholesterol supply. Possible explanations of statin-induced inhibition of proliferation may involve decreased isoprenylation and depletion of dolichol, a substance essential for N-glycosylation, which is required for maturation of insulin receptors and type 1 IGF receptors. Since insulin and IGFs induce proliferation of TI cells and since TI cells may express IGFs, we proposed that statins inhibit proliferation, at least in part, by decreasing N-glycosylation and thus limiting the availability of mature insulin/IGF receptors. To test this hypothesis, we studied the effects of inhibition of glycosylation on proliferation of TI cells.

Methods: Rat TI cells were cultured for 48 hours in the absence (control) or in the presence of simvastatin (1-30μM), farnesyl pyrophosphate (FPP; 0.3-10μM), geranyl-geranyl pyrophosphate (GGPP; 0.3-10μM) or inhibitors of glycosylation: tunicamycin (TUN, 10-100ng/ml), castanospermine (CAS; 3-30μg/ml) and deoxymannojirimycin (DEO, 0.1-1mM). The data were analyzed by ANOVA and post-hoc comparisons.
Results: Simvastatin induced dose-dependent inhibition of proliferation down to 11% ($P<0.001$) of control level. This effect of simvastatin was only partly reversed in the presence of FPP or GGPP, resulting in proliferation, respectively, at 31% and 18% of control. All glycosylation inhibitors blocked proliferation in a concentration-dependent fashion. TUN decreased proliferation by up to 99%, CAS by up to 43%, and DEO by up to 39%; all these effects were significant at $P<0.001$.
Conclusion: Inhibition of proliferation by simvastatin is only partly explained by blocked isoprenylation. Since proliferation is sensitive to diverse inhibitors of glycosylation, we postulate that statins may act in part by blocking N-glycosylation. Furthermore, the present findings indirectly support the concept that autocrine/paracrine regulation of TI proliferation may involve the IGF system.
Support: NIH grant R01 HD 40207.

793

Evidence for Primordial Follicle Regeneration Post-Chemotherapy in Human Ovarian Xenografts. Kutluk Oktay,* Ozgur Oktem. *Department of Obstetrics & Gynecology, Weill Medical College of Cornell University, New York, NY, USA.*
Background & Rationale: Recent work has suggested that oocyte regeneration can occur in adult mice. Moreover, there have been numerous reports of return of fertility in women who were rendered menopausal by high dose chemotherapy. Stem cell side populations have been reported in nearly all adult organ systems. We thus hypothesized that ovarian side populations can result in primordial follicle (PF) regeneration in response to acute follicle loss induced by chemotherapy. To test this hypothesis, we used a fetal human ovarian xenograft model previously developed by us. **Methods:** Fetal ovaries of 23-24 wk gestation contain a nearly pure population of PF. 9 mm^3 ovarian pieces were subcutaneously grafted to SCID mice with matrigel. After 2 wks, the animals (n=52) received either 200 mg/kg cyclophosphamide (Cy) or the vehicle, and were sacrificed at 12-72h post-injection (n=6-7/group). Xenografts were serially sectioned and PF numbers/mm^2 (PFD)were determined. **Results:** While the PFD remained steady from 12-72h post injection in the controls, follicle numbers sharply declined until 48h post chemo injection. Interestingly, PFD increased at 72h (fig). To ascertain that this recovery in PFD is not due to regeneration of PF from pre-existing fetal mitotic oogonia, we stained adjacent sections for mouse vasa homologue (MVH) and a mitosis marker phospho-histone-H3. The number of cells staining with both markers/mm^2 was compared to the staining in sections from a 22-year old female. Both had very few cells doubly-staining, ruling out the possibility of regeneration from mitotic germ cells. In fetal xenografts we also observed MVH positive germ cells migrating well in to the matrigel matrix encapsulating the grafts.
Conclusions: Primordial follicle regeneration may be induced in "mature" fetal ovarian tissue subjected to genotoxic stress such as chemotherapy. If these observations are confirmed in postnatal tissue, the mechanism of recovery of ovarian function following chemotherapy-induced ovarian failure can be explained. The finding of germ cell migration from xenografts deserves further investigation.



794

Decreased Fertility Following Fetal and Neonatal Exposure to Nicotine May Be Explained by Impaired Ovarian Angiogenesis. Alison C Holloway,*[1] James J Petrik.[2] [1]*Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada;* [2]*Biomedical Sciences, University of Guelph, Guelph, ON, Canada.*
Introduction: It is well documented that cigarette smoking is associated with a number of adverse obstetrical outcomes yet 15-20% of all pregnant women smoke. In human populations there is evidence that *in utero* exposure to cigarette smoke has long-term consequences on the fertility of the female offspring. We have shown that fetal and neonatal exposure to nicotine results in impaired fertility in adulthood and propose that this is due to altered ovarian angiogenesis. The ovary is a unique structure as it is one of the few adult tissues that continually undergo angiogenic processes. Numerous studies have demonstrated that normal vessel formation is essential for follicular and luteal development, and any alteration in ovarian angiogenesis can have a significant influence on reproduction.
Objective: To determine if reduced fertility in adult rats exposed to nicotine during fetal and neonatal development can be attributed to altered ovarian angiogenesis.
Methods: Maternal rats were exposed to nicotine (1mg/kg/d) for 2 weeks prior to mating until weaning. Ovaries were collected from saline and nicotine treated rats at PND1, at 4, 16 and 32 weeks of age and fixed in 10% neutral buffered formalin. Immunohistochemistry was performed for CD31 as a marker for vessel endothelial cells and vascular endothelial growth factor (VEGF). For measurements of vascularization, microvessel density (MVD; the total count of microvessels per optical field) and total vascular area (TVA; the total area occupied by microvessels per optical field) were quantified. VEGF staining was quantified as the percentage of immunopositive ovarian tissue in 6 different fields of view.
Results: At PND1, there was no difference in MVD or TVA in ovaries from saline or nicotine treated dams. Similarly, no change was seen in VEGF expression at PND1. However, by postnatal week 4, there was a significant decrease in MVD, TVA and VEGF expression in ovaries from nicotine-treated dams, as compared to saline-treated controls. At postnatal weeks 16 and 32, ovaries from offspring from nicotine-treated dams continued to have reduced blood vessel density and ovarian VEGF expression.
Conclusion: The results from this study suggest that one mechanism by which maternal cigarette smoke may impair the fertility of the female offspring is through alteration of ovarian angiogenesis.

795

Infusion of Intermedin Antagonist (IMD_{17-47}) Causes a Decline in the Levels of Estrogen and Progesterone in Pregnant Rats. Madhu S Chauhan, Rebekah Elkins, Chandrasekhar Yallampalli.* *Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Intermedin (IMD) is a recently discovered peptide which belongs to calcitonin/CGRP peptide family. Analysis of Intermedin expression profile has shown that transcripts of Intermedin and its receptor complexes are expressed in ovarian granulose cells suggesting that Intermedin may play a role in the regulation of ovarian function. Further, our recent studies using IMD antagonist, IMD_{17-47}, showed decreases in placental and fetal growth as well as apoptotic changes in IMD_{17-47} infused rat placenta. Therefore, we hypothesize that IMD may be involved in regulating sex steroid hormone synthesis in rat pregnancy.
OBJECTIVES: 1) To determine the effect of IMD_{17-47} infusion on plasma levels of estradiol (E_2) and progesterone (P_4)in day 9 pregnant rats and 2) to assess the IMD_{17-47} induced changes on the expression of enzymes involved in ovarian steroidogenisis in the ovaries collected from rats infused with IMD_{17-47} and the controls.
METHODS: Sprague Dawley rats were used in this study. Osmotic mini-pumps containing vehicle alone or IMD_{17-47} (200 μg/day) were inserted s.c. in rats on day 3 of gestation and sacrificed on day 9 (n = 5). Blood samples were collected in tubes containing aprotinin and spun at 600 X g for 10min at 4^0C. Plasma collected was stored at -80^0C until used for Radioimmunoassay to analyze estradiol and progesterone levels. Total RNA was isolated from ovaries using TRIzol reagent and processed for RT-PCR of various steroidogenic enzymes and the results are expressed relative to 18S mRNA.
RESULTS: Our results demonstrate that, 1) IMD antagonist caused a significant decline in the levels of both estradiol and progesterone ($p<0.05$), 2) IMD_{17-47} infusion caused a significant increase in the mRNA levels of StAR, but has no effect on the levels of SCP2 mRNA and; 3) IMD_{17-47} caused a significant increase ($P< 0.05$) in the mRNA levels of p450scc, p450c17 (CYP17), p450arom, 20b-HSD, 3b-HSD and 17b-HSD.

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CONCLUSION: IMD_{17-47} causes a decline in the levels of both progesterone and estradiol suggesting a role for IMD in the regulation of circulating sex steroid hormones during early rat pregnancy. Because IMD_{17-47} increased steroidogenic enzyme levels in the treated ovaries, the decline in the steroid hormone levels observed suggest a possible effect of IMD_{17-47} on the rate of clearance of the E_2 and P_4 from the blood.

796

Renin-Angiotensin-Aldosterone System (RAAS) in Human Ovaries. Rita M Sneeringer,[1,2] Jennifer Eaton,[1] Yevgeniya Monisova,[3] Alan Penzias,[*1,2] Anny Usheva.[3] *[1]Ob/Gyn Reproductive Endocrinology & Infertility, Beth Israel Deaconess, Boston, MA; [2]Boston IVF, Waltham, MA; [3]Endocrinology, Beth Israel Deaconess, Boston, MA.*

INTRODUCTION: Ovarian RAAS has been proposed to exist, but the presence of aldosterone and mineralcorticoid pathway constituents and their relation with the maturation of fertility competent oocytes has not been demonstrated.

METHODS**:** Human follicular fluid (FF) from large (Lf) and small (Sf) follicles obtained 36 hours post human chorionic gonadatropin from 28 IVF patients was analyzed for aldosterone (Ald), corticosterone (CS), and angiotensin 1 (Ang1) content by ELISA. Concentrations were compared with normal blood levels and correlated with age, number of retrieved and mature oocytes (SPSS software). Differences between Lf (>14mm) and Sf (<10 mm) were compared (t-test).

RESULTS: FF from Lf had significantly increased mean concentration of CS (1.70E+5 pg/mL vs. 1.11E+5 pg/mL, p=0.23) and a trend for increased Ald concentration (488 pg/mL in Lf vs. 416 pg/mL in Sf, p=0.60). No difference was observed in Ang1 content (226 pg/mL vs. 232 pg/mL, p=0.921). Ald and CS concentrations in FF were much greater than normal blood concentrations (CS 900-3900 pg/ml and Ald 25-315 pg/ml). CS concentration was positively correlated with the number of oocytes retrieved (R2=0.413, p=0.45) and the aldosterone concentration (R2=0.521, p=.016). Ang1 was negatively correlated with age (R2=-0.559, p=0.38).

CONCLUSIONS: This is the first demonstration of the mineralcorticoid pathway presence within human ovarian FF. High concentrations of pathway constituents suggest possible local synthesis. Correlations with the number of oocytes retrieved suggest possible RAAS involvement in maturation of fertility competent oocytes. The precise function is under investigation.

FF Mineralcorticoids / RAS

	CS	Ald	Ang1
Large FF	169,930 (83,825)	488 (94)	226 (140)
Small FF	111,185 (97,460)	417 (139)	233 (198)
P value	0.023	0.060	0.921

Concentrations = Mean (Standard Deviation) pg/mL; Reference plasma values: CS 900-3900 pg/mL and Ald 25-315 pg/mL

FF Mineralcorticoids and Fertility Factors

	Age	#Oocytes	#Mature Oocytes
CS	-0.203 (0.300)	+0.428 (0.023)*	+0.345 (0.072)
Ald	-0.146 (0.527)	+0.153 (0.508)	+0.84 (0.718)

Pearsons Coefficient (Significance); *Statistically significant; Data for large follicles only (similar trends with small follicles)

797

Potential Role of the Cholesterol Trafficking System in Human Follicular Maturation and Fertility Competence. Jennifer L Eaton,[1] Yevgeniya Monisova,[1] Rita Sneeringer,[1,2] Alan Penzias,[*1,2] Richard Reindollar,[*3] Anny Usheva.[4] *[1]Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston, MA, USA; [2]Boston IVF, Waltham, MA, USA; [3]Obstetrics and Gynecology, Dartmouth Hitchcock Medical Center, Lebanon, NH, USA; [4]Medicine, Harvard Medical School, Boston, MA, USA.*

INTRODUCTION: The cholesterol (Ch) trafficking system in human ovarian follicular fluid (FF) is not well understood.

OBJECTIVE: Establish the relationships between aging, follicle maturation, and content of Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB), triglycerides, and total Ch in FF.

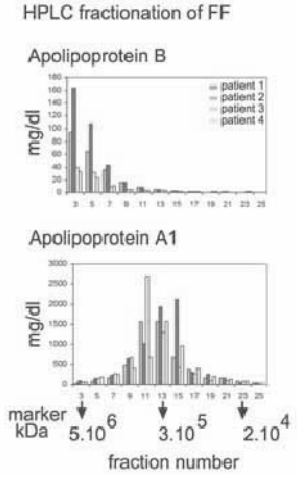
METHODS: FF was collected from small (<11mm) and lead (>16mm) follicles from old (>40 years) and young (<34 years) IVF patients. ApoA1 and ApoB were analyzed by enzyme-linked immunosorbent assay (ELISA). Total Ch and triglycerides were determined enzymatically. Presence of lipoprotein complexes was determined by gel filtration HPLC.

RESULTS: ApoB concentration was significantly greater in small follicles than in lead follicles (P = 0.04). There were trends toward increased ApoB and decreased ApoA1 content in old versus young patients. There was no clear difference in ApoA1 concentration between small and lead follicles. ApoB was observed primarily in HPLC fractions 3-7, while ApoA1 was detected mainly in fractions 11-13. Total Ch was greater in small follicles than in large follicles (81 vs. 69 mg/dl, P=0.03). Triglycerides were not detected in FF.

CONCLUSIONS: This is the first demonstration of the presence of ApoB and ApoA1 together in human FF. ApoB forms large complexes that may contain cholesterol. ApoA1 is present in complexes with unknown factors, as evidenced by HPLC. Correlations with follicular size and age suggest possible involvement in maturation and fertility competence. The precise function is under investigation.

ApoA1 and ApoB Content

		Young (n=16)	Old (n=12)	Total
ApoB (mg/ml)	Small (n=8)	10.03 +/- 8.47	19.85 +/- 0.57	12.48 +/- 8.48
	Lead (n=8)	3.24 +/- 2.13	9.34 +/- 8.36	4.77 +/- 4.60
ApoA1 (mg/ml)	Small (n=8)	9.49 +/- 2.91	8.37 +/- 0.72	9.49 +/- 2.91
	Lead (n=8)	9.13 +/- 2.54	7.58 +/- 0.55	8.75 +/- 2.28



798

Detailed Ultrasound Real-Time Velocity Profiles of Ovarian Circulation along Menstrual Cycle Using a Novel Global Acquisition & Signal Processing (G.A.S.P.) Software for the Multigate Spectral Doppler Analysis: A Prospective Study. Gabriele Urban,[1] Michael J Paidas,[2] Stefano Ricci,[3] Piero Tortoli,[3] Fabio Sanguineti,[2] Charles J Lockwood,[*] Pasquale Patrizio. *[1]Obstetrics and Gynecology, University of Milan Bicocca, Monza, Italy; [2]Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [3]Electronics and Telecommunication, University of Florence, Florence, Italy.*

Objective To study, using a new multigate Doppler system, the uterine artery, external iliac artery and vein, hypogastric artery and vein, ovarian artery and vein blood flow velocities profile in real-time.

Methods The Multigate Spectral Doppler Analysis (MSDA) system employed in this study consisted of commercial ultrasound machine (Aloka SSD1400) connected to personal computer (PC) where a proprietary electronic board was plugged and where the G.A.S.P software ran. Imaging was performed within standard safety guidelines. We scanned left and right uterine arteries, the external iliac artery and vein, hypogastric artery and vein, ovarian artery and vein in 42 women at day 5-10 and 15. In post processing we evaluate velocities profiles, wall distension rate and wall shear rate.

Results We were successfully able to generate a velocity profiles from all the vessels interrogated from both sides, remarking different flow directions in real-time. Qualitative description of flow is consistent with the expected laminar flow pattern of those vessels. Comparables conventional Doppler spectrums corresponds to different velocity profile in systole like in diastole. Velocity profile are consistent in the same patients. Systolic and diastolic maximum and mean velocities discretes and value interpolations were calculated

Conclusions We found a substantial difference between conventional Doppler results and velocity profile from MGSD. Qualitative description of flow show the complete distribution across the lumen instead of cross-sectional mean which preserve his profile during consecutive cycles. Our study seem support the hypothesis that increasing resistance during ovarian cycle are mainly due by f blood viscosity increases.

ovarian cycle	WSR	rWDR	VP	cycle day correlation index
Hypogastric	455±217*	2.5±0.5	4	0.883
Uterine	637±260*	1.7±1.0	5	0.8
Ovarian	377±130*	2.4±1.7	3	0.7

*p<0.01

799

Stem Cell Transplantation Prevents Age-Related Infertility in Adult Female Mammals. Kaisa Selesniemi, Ho-Joon Lee, Jonathan L Tilly.* *OB/GYN, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.*

Introduction: Recent studies showed that bone marrow (BM) transplantation (BMT) restores oocyte production in chemically- or genetically-sterile female mice (*Cell* 2005 122:303-15). Subsequent studies with adult male mice produced comparable data in that spermatogonia could be derived from BM (*Lab Invest* 2006 86:654-63). Our unpublished findings from long-term mating trials have further shown that BMT rescues fertility and fecundity in chemotherapy-treated young adult female mice. **Objective:** Herein we tested if serial BMT, in the absence of any recipient conditioning, could delay or reverse age-related infertility in females. **Methods:** Wild-type female mice at 8 months of age were given a single tail vein injection of vehicle (n=12) or BM (n=12), and this was repeated monthly for the duration of the study. For transplantation, BM was harvested from young GFP-transgenic females. Mating trials were started at 10 months of age, and continued for 3 successive trials allowing the offspring to reach weaning before the next trial. Since about half of the female mice will be infertile by 10 months of age, this study design allowed us to test if BMT could prevent the onset of infertility in reproductively competent animals and reverse infertility in animals that had already undergone reproductive senescence. **Results:** In the first trial, pregnancy success rates were comparable in mice receiving vehicle (50%; 6/12) or BM (58%; 7/12). While pregnancy success rates in the second trial remained unchanged in control mice (50%; 6/12), 75% (9/12) of the mice receiving BMT achieved a successful pregnancy. By the end of third trial, pregnancy success rates dropped to 27.3% (3/11) in the control group, whereas 58% (7/12) of the mice receiving BMT achieved successful pregnancies. Fecundity (pups/litter) in the BMT group (5.1 ± 2.3) was also higher than controls (3.7 ± 2.1). Finally, the same females in each trial delivered pups, and all pups were derived from the recipient germline. **Conclusions:** Age-related female infertility can be delayed by transplantation with adult stem cells derived from BM. Since the same females retained the ability to become pregnant and deliver offspring, BMT is most effective at preventing, rather than reversing, infertility in aging females. Further, the ability of BMT to sustain fertility with age appears independent of germline cells in the transplants since all offspring were host-derived (Support: NIH R37-AG012279).

800

The Molecular Profile of Stromal Side Population in Human Endometrium Has the Features of a Progenitor Stem Cell Population. Irene Cervello,[1] Alicia Martinez-Romero,[2] Jose Antonio Martinez-Conejero,[1] Sebastian Martinez-Escribano,[1] Jose Antonio Horcajadas,[1] Antonio Pellicer,*[1] Jose Enrique Oconnor,[2] Carlos Simon.*[1,3] *[1]Fundacion IVI, Instituto Universitario IVI-University of Valencia, Valencia, Spain; [2]Citomycs Laboratory, Unidad Mixta CIPF-UVEG, Valencia, Spain; [3]Valencia Stem Cell Bank, Unidad Mixta CIPF-UVEG, Valencia, Spain.*

Objectives: The endometrial adult progenitor stem cell (PSC) population has been recently described by different groups; however their characterization has not been yet achieved. The aim of the present study was the isolation and molecular characterization of the stromal side population (SP) in the human endometrium.

Materials and Methods: Human endometrium from healthy donors was obtained after writing informed consent for this project. The stromal fraction was isolated using an established protocol with minor modifications (JCEM.,1993). Purity of the stroma cell suspension was assessed by vimentine expression using a Cytomics FC500 flow cytometer (Beckman-Coulter, CA, USA). Isolated stromal cells were incubated with bis-benzimide Hoechst 33342 dye and Hoechst 33342 dye+verapamil (negative control) during 90-120 min at 37ºC. Further, propidium iodide was added before flow-cytometric sorting to exclude dead cells. SP (low Hoechst-stained cells) and Non-SP (stained cells) were separated with a MoFloÒ (Dako,CO,USA) sorter and resuspended in serum media.

Both cell populations were analyzed for telomerase activity, nested-PCR for OCT4, c-Kit and ABCG2 (also know BCRP1). Moreover immunocytochemistry of the SP was performed for Bcrp1 analysis.

Results: Approximately 80% of the cell population analyzed by flow cytometry corresponded to the stromal compartment. Stromal SP effluxed the vital dye and co-treatment with verapamil blocked the ABC transporter inhibiting Hoechst extrusion. They represented 0.13%± 0.03% of the total stromal compartment analyzed. Cells from SP and Non-SP were separated and analyzed to further determine their molecular profile. Immunocytochemistry demonstrated that 97.83% of cells were positive to the Bcrp1 marker. At the mRNA level, Bcrp1, OCT4 and c-Kit expression were detected by nested-PCR in the SP population.

Conclusions: We have isolated and characterized the stromal SP molecular profile of human endometrium. These results strongly suggest that the SP obtained by flow cytometry has the features of a PSC population in the human endometrium.

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801

Proliferation, Differentiation, and Cytokine Secretion of Cultured Human Umbilical Cord Blood-Derived Mononuclear Cells. Sandra Neuhoff,[1] Janet Moers,[1] Arne Jensen,*[2] Rolf Dermietzel,[1] Carola Meier.[1] *[1]Neuroanatomy and Molecular Brain Research, Ruhr-University Bochum, Bochum, Germany; [2]Gynecology and Obstetrics, Ruhr-University Bochum, Bochum, Germany.*

Objective: Intraperitoneal transplantation of human umbilical cord blood (hUCB)-derived mononuclear cells led to the specific 'homing' of these cells to a hypoxic-ischemic lesion in perinatal rats. Motor deficits resulting from the lesion were alleviated upon transplantation (1). However, the molecular and cellular mechanisms underlying the observed functional improvements are still unclear. As neuronal differentiation of transplanted cells seems to be a rare or absent event in vivo, we propose secondary mechanisms, which might be responsible for the therapeutic effects. One possibility is that transplanted cells might contribute to a regenerative environment by secretion of cytokines.

Methods: Using a succession of distinct culture media, mononuclear cells were stimulated by growth factor combinations, i.e. epidermal growth factor / fibroblast growth factor-2 or nerve growth factor / retinoic acid, and analyzed immunocytochemically. Conditioned medium was collected at various time points and subsequently assayed for levels of secreted proteins by using a Human Cytokine Antibody Array.

Results: HUCB-derived mononuclear cells in culture responded to growth factor treatment with proliferation, as assessed by detection of the Ki-67 protein. In addition, neural differentiation was initiated, demonstrated by expression of neuronal and glial marker proteins. Most importantly, in response to either growth factor combination, cells were shown to secrete significant levels of various cytokines, including several interleukins, growth factors, and chemotactic proteins.

Conclusion: Although capable of incipient differentiation, cytokine secretion of hUCB-derived mononuclear cells envisages the potential of an indirect effect in vivo. Most factors detected in conditioned medium are renowned for their anti-inflammatory, neuroprotective, angiogenic, or chemotactic action. These results are promising in that secreted cytokines of human mononuclear cells might be suitable candidates mediating functional recovery after hypoxic-ischemic brain injury.

(1) Meier et al. (2006) Pediatr. Res. 59:244-249

802

Expression of Germline Specific Markers in Adult Murine Ovarian Cell Aggregates. Dong Zhang, Hala Fouad, Willie Zoma, Salama Abdou Mohamed Salama, Melissa J Wentz, Ayman Al-Hendy.* *Dept of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Introduction: Reproductive biology dogma refuting postnatal mammalian oogenesis was challenged recently by reports indicating that germline stem cells generate new follicles in adult murine ovary. Oct4 is a POU family transcriptional regulator restricted to embryonic germ cells and embryonic stem cells. Stem cells factor receptor (SCF-R) expressed mainly during early oogenesis. Stage specific embryonic antigen 1(SSEA1) is expressed in primordial germ cells. Mouse vasa homologue (MVH) is a specific surface marker of mammalian germ cell line. The aim of present study was to investigate the expression and distribution pattern of these cell surface markers in adult mouse ovary.

Methods: Ovaries of B6.CBA-Tg(pou5f1-EGFP) transgenic female mice aged 8-12 weeks were harvested, digested and EGFP(+) cell aggregates were isolated under fluorescent microscopy. Cell aggregates were cultured for three days and prepared for both H & E and immunohistochemical staining with antibodies against SSEA1, MVH, and SCF-R. Total RNA was extracted from these EGFP(+) aggregates and RT-PCR performed to assess the expression of germ cell and stem cell markers.

Results: Two types of EGFP(+) cell aggregates were identified in the ovaries: large aggregates representing mature ovarian follicles and small distinct cellular aggregates lacking any follicular pattern distributed in the periphery of the ovaries (about 15-20 isolated from each ovary). Most of the cells within the smaller aggregates co-expressed EGFP with SSEA1, MVH and SCF-R. RT-PCR on the small cell aggregates confirmed expression of Oct4, MVH, SCF-R as well as meiotic markers (SCP3 & DMC1), with no expression of the mature oocyte marker (GDF9).
Conclusion: Our study showed that OCT4-positve cell aggregates co-expressing germline and stem cell surface markers exist in adult murine ovary. These cell aggregates may represent a mixed population of germline stem cells and developing germ cells. Further research is underway to evaluate the potential plasticity of these cells.

803

Engraftment Potential of Human Placenta-Derived Mesenchymal Stem Cells after In Utero Transplantation in Rats. Chie-Pein Chen,[1,2] Shu-Hsiang Liu,[2] Jian-Pei Huang,[1] Ming-Yi Lee,[2] Pei-Chun Chen,[2] Cing-Siang Hu,[2] Yi-Hsin Wu,[2] Chun-Chuan Ko,[2] Yuh-Cheng Yang.[2] (SPON: Philip Newton Baker). *[1]Division of High Risk Pregnancy, Mackay Memorial Hospital, Taipei, Taiwan; [2]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan.*
Objective: Human placental mesenchymal stem cells (hPMCs) are thought to be multipotent. However, it is unknown what their fate would be if they are transplanted in utero. In this study, we aimed to establish a rat model for in utero transplantation of hPMCs to investigate if these cells underwent in utero transplantation would result in long-term, organ-specific engraftment.
Methods: hPMCs were isolated from term placentas and assessed for phenotype markers and mutilineage capacity in vitro. The immunomodulatory properties of the cells were studied in xenogeneic mixed lymphocyte reactions. Their engraftment potential was analyzed in a pregnant rat model after in utero transplantation of hPMCs into the peritoneal cavity of fetus through transuterine injection after laparotomy at embryonic day (E) 17. Immunohistochemistry, cell-labeling tracing, and human X-chromosome specific fluorescent in situ hybridization were used to assess post-transplant chimerism.
Results: In vitro, lineage-negative, CD34-negative hPMCs differentiated into osteocytes, adipocytes, and endothelial cells with tube formation. After in utero transplantation into pregnant rats, the cells engrafted in various fetal tissues originating from all three germ layers with site-specific differentiation and persisted for at least 12 weeks after delivery. Engraftment occurred in more than 80% of the fetal rats. hPMCs actively suppressed lymphocyte proliferation responsiveness induced by allogeneic lymphocytes. Significant numbers of cells engrafted in the fetal lung when assessed at E21. The cells retained the ability to proliferate.
Conclusion: We conclude that hPMCs are mutipotent cells that can be engrafted long-term in immunocompetent rats after in utero transplantation. hPMCs thus may be an alternative cell source for in utero fetal therapy.

804

Co-Culture Effects on Trophoblast Differentiation from Human Embryonic Stem Cells. Maria Giakoumopoulos,[1,2] Leah M Siegfried,[1,2] Mark A Garthwaite,[1,2] Thaddeus G Golos.[1,2] (SPON: Ronald R Magness). *[1]National Primate Research Center, University of Wisconsin, Madison, WI, USA; [2]Obstetrics and Gynecology, University of Wisconsin, Madison, WI, USA.*
Objective: Our laboratory has recently shown that when human embryonic stem cells (hESC) are allowed to differentiate under specific conditions in culture, they will differentiate into trophoblasts and secrete high levels of placental hormones. We therefore propose a three-dimensional, co-culture system to achieve the goal of utilizing various effector cell types to drive differentiation.
Methods: We used GFP-expressing H1 human embryonic stem cells (H1EGFP hESC) in combination with red Cell Tracker-labeled human term placental fibroblasts (TPF) and chorionic villus sampling fibroblasts (CVS) to form embryoid bodies (EBs). These EBs were kept in culture, in suspension for 30 days with media collection and replenishing done daily on half the media.
Results: Levels of human chorionic gonadotropin (hCG) in combination (TPF/H1EGFP) EBs in suspension increased 3-fold by day 20, peaked by day 25 (244.5 ng/ml) and dropped again by day 30, compared with hESC-only EBs, that maintained a low level of secretion (<19.1 ng/ml). CVS/H1EGFP combination EBs increased earlier at day 15, dropped by day 20 and then peaked at day 30. Immunohistochemistry of both combination EB types confirmed trophoblast differentiation by cytokeratin and hCG staining.
Conclusions: Effector cells can facilitate trophoblast differentiation through cell to cell contact with hESC within aggregated EBs. Furthermore, the three-dimensional suspension environment fosters a greater hCG secretion than is seen in hESC-only EBs.

805

Nestin, a New Marker for Differentiation of Endothelial Progenitor Cell in Human Umbilical Cord Blood. Han-Sung Hwang,[1] Young-Guen Kwon,[2] Yong-Sun Maeng,[2] Han-Sung Kwon,[3] Yong-Won Park,[1] Young-Han Kim.[1] (SPON: Brian J Koos). *[1]Obstetrics and Gynecology, Yonsei University College of Medicine, Seoul, Democratic Peoples Republic of Korea; [2]Biochemistry, Yonsei University, Seoul, Democratic Peoples Republic of Korea; [3]Obstetrics and Gynecology, Konkuk University, School of Medicine, Seoul, Democratic Peoples Republic of Korea.*
Objective: Nestin, a type VI intermediate filament protein has been originally described as a marker of neural stem or progenitor cells, and represents the proliferation capacity of the cells. Recent reports have documented nestin expression in the endothelium of proliferating or newly formed blood vessels. The aim of this study was to assess the expression of nestin gene in endothelial progenitor cell (EPC) obtained from umbilical cord bloods and outgrowing endothelial cell (OEC) obtained after EPC differentiation.
Methods: In umbilical cord bloods obtained from women undergoing cesarean section with normal pregnancies at term, mononuclear cells were isolated by density gradient centrifugation and cultured on 6-well plates coated with human fibronectin in endothelial growth medium 2 (EGM-2). We performed double staining for both Dil-acetylated low-density lipoprotein and Ulex europaeus agglutinin-FITC for identification of EPC from the attached mononuclear cells after 7 days in culture. Identified EPCs were differentiated into OECs after 10 days in culture. Total RNA was obtained from EPCs and OECs. After gene expression of cell markers known in EPC and OEC was identified by semi-quantitative RT-PCR, the nestin gene expression was detected in each cell by semi-quantitative RT-PCR and microarray.
Results: Microarray and semi-quantitative RT-PCR revealed that nestin gene was downregulated in EPC, but upregulated in OEC.
Conclusions: Our studies showed that nestin could be used as a new marker of EPC differentiation. Furthermore, our data suggest that nestin may be a promising tool for estimating proliferation and neovascularization capacity of fetal endothelial cell.

806

Neurogenic Potential of Placenta-Derived Mesenchymal Stem Cells. C Bettina Portmann-Lanz, Andreina Schoeberlein, Ruth Sager, Alexander Huber, Henning Schneider,* Daniel V Surbek. *Obstetrics and Gynecology, University Hospital of Berne, Berne, Switzerland.*
Objective :We have recently shown that mesenchymal stem cells (MSCs) exist in extrafetal placental tissue and that these cells can not only enter the mesodermal but also the neurogenic lineage. The aim of this study was to determine the neuronal, oligodendrocytic and astrocytic fate of placental MSCs under certain neurogenic culture conditions.
Methods: We isolated fetal MSCs from first trimester (8-14 weeks) placental chorionic villous stroma and term gestation (>37 weeks) chorion. Differentiation towards the neuroectodermal lineage was initiated by treating with retinoic acid (RA) for different time intervals (1-6 weeks). Differentiation into neurons, oligodendrocytes and astrocytes as well as their progenitors was assessed immunohistochemically using stage specific markers.
Results: Untreated placental MSCs already contained 7% of Tuj-1 positive cells. The number of these very early, immediately postmitotic neurons increased 4-5x after exposing 1 week to RA. Tuj-cells progressively decreased with continuing RA-treatment up to 6 weeks and at this time about 1% MAP-2 positive and NeuN negative postmitotic neurons were identified. Unstimulated cells contained about 30% of very early immature O1-positive oligodendrocytes which disappeared within a few days after RA stimulation. After 3-6weeks about 3% of the cells were positive for O4, NG2 or MBP, meaning that by RA treatment only a few cells are able to differentiate towards more mature resp. mature oligodendrocyte forms. With our methods we could not detect astrocytes or progenitors in control cells and after 1-3 weeks of RA-stimulation. However, after 6 weeks, a few cells (1%) were expressing GFAP and GDNF, sometimes also FGF3 R3 and GLNS. Neuronal, oligodendrocytic and astrocytic differentiation initiated by RA seems not to depend on gestational age resp.the percentage of cells differentiating towards a certain neurogenic lineage varied not significantly between chorionic villous MSCs from first trimester compared to chorionic MSCs from term placentas.

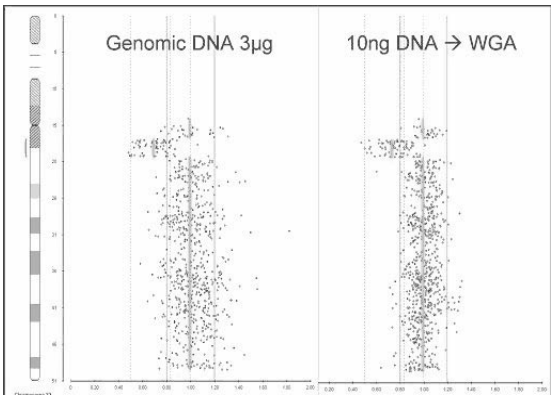
Conclusion: Placental MSCs enter the neurogenic lineage adopting mainly neuronal and oligodendrocytic fate. MSCs, obtained from placental tissue either during CVS or at delivery can differentiate into early neural progenitors and might be an ideal source for autologous stem cell graft for peripartum neuroregeneration.

807

Trophoblasts Induce Endothelial Progenitor Cells Proliferation. Emiko Abe, Keiichi Matsubara, Masaharu Ito. (SPON: Ronald R Magness). *Obstetrics and Gynecology, Ehime University School of Medicine, Toon, Ehime, Japan.*
Objectives: It is reported that bone marrow derived endothelial progenitor cells (EPCs) were isolated from circulating mononuclear cells in human peripheral blood. EPCs are involved in neovascularization,not only fetal period (vasculogenesis) but also postnatal neovascularization. In early pregnancy, the abundant placental vascularization leads to the formation of uteroplacental circulation. We hypothesized that trophoblasts stimulated the proliferation of placental neovascularization.
Methods: Informed consent was obtained from each patient. Bone marrow cells obtained from green fluorescent protein (GFP) transgenic mice were injected into irradiated female NOD/SCID mice and they were mated. On the 1st and 3rd gestational weeks, the uteri were obtained and the GFP positive cells' distribution was examined under fluorescent microscopy. Peripheral mononuclear cells (PMNs) from women in their late luteal phase were cultured with or without trophoblasts respectively, from women in gestational week 6~9. After 7 days, the number of EPCs was counted in terms of the intake of both LDL and lectin by using flow cytometry. The concentration of PlGF of sera cultured with or without trophoblasts were also measured. To examine the migration of EPCs, we measured EPCs' mobilization induced by humoral factors from trophoblasts, using a live cell culture imaging system. Statistical analysis was then performed using one-way ANNOVA.
Results: GFP positive cells were observed around the embryo in the 1st gestational week. The number of EPCs in culture with trophoblasts was found to be significantly increased (5737±1157 cells/well, mean±SE) compared to that without trophoblasts (2587±503, $p<0.05$). The concentration of PlGF cultured with trophoblasts was also significantly higher (165.7 ±16.8 pg/ml) compared to those cultured without trophoblasts (3.15±0.76, $p<0.05$). However, humoral factors from trophoblasts did not affect the EPCs' mobilization.
Conclusions: Trophoblasts have the potential to stimulate placental vasculogenesis proliferation, rather than EPC mobilization.

808

Oligonucleotide Array Based Comparative Genomic Hybridization with Limited Amounts of DNA Using Whole Genome Amplification. Jessica B Spencer,[1] Elijah J Wallace,[2] Amy Kogan,[2] Donna Session,[*1] David H Ledbetter,[2] Christa Lese Martin.[2] *[1]Dept of Gynecology and Obstetrics; [2]Dept of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA.*
Objective
The development of commercially available whole genome amplification (WGA) kits has made it possible to detect genome-wide copy number abnormalities using oligonucleotide microarrays with relatively limited amounts of starting DNA. The purpose of this study was to determine the sensitivity of the array in detecting these changes using comparative genomic hybridization (CGH) after WGA. Various amounts of starting DNA were tested with the ultimate goal of using a single cell.
Methods
Genomic DNA from a female with a FISH confirmed 3 Mb deletion of 22q11 (DiGeorge syndrome region) was utilized as the test sample. Array analysis was performed using three different starting genomic DNA concentrations: 3ug, 10ng and 100pg. The GenomiPhi v2 kit (GE), which utilizes random hexamer primers with bacteriophage Phi29 DNA polymerase, was used for WGA. The 10ng and 100pg test samples were prepared with the WGA protocol (final yield 3-5ug) while the 3ug starting template was hybridized directly. Sample preparation and array hybridization followed manufacturer's instructions (Agilent 44B protocol v4.0). All samples were hybridized for at least 40 hrs and analyzed with BlueFuse v3.4 (BlueGnome).
Results
All three arrays successfully detected the 22q deletion. The WGA 10ng array detected the exact same region as the unamplified genomic DNA with very limited background.



The 22q deletion was also detected with the 100pg array, however, there was a significantly higher background.
Conclusions
Array CGH offers a method for higher resolution copy number detection than traditional cytogenetic analysis, which will likely miss abnormalities <5MB in size. We have shown that as little as 10ng of starting DNA, such as might be encountered from very early pregnancy loss or difficult chorionic villous sampling, can still be accurately analyzed with this microarray. We are continuing to optimize this protocol for picogram amounts of DNA such as those obtained from single cells for PGD.

809

Birth and Death of Galectins Predominantly Expressed in Placenta. Nandor Gabor Than,[1] Roberto Romero,[*1,2] Derek E Wildman,[1,2] Adi L Tarca,[1,3] Offer Erez,[1] Yu Mi Han,[1] Sung-Su Kim,[1] Amy Weckle,[1] Monica Uddin,[2] Enola Cushenberry,[1] Jimmy Espinoza,[1,4] Chong Jai Kim,[1] Sorin Draghici,[3] Zolton Papp,[5] Lawrence I Grossman,[2] Joaquin Santolaya-Forgas,[*6] Morris Goodman.[2] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Molecular Medicine & Genetics, Wayne State University, Detroit, MI, USA; [3]Department of Computer Science, Wayne State University, Detroit, MI, USA; [4]Department of Obstetrics & Gynecology, Wayne State University, Detroit, MI, USA; [5]Department of Obstetrics & Gynecology, Semmelweis University, Budapest, Hungary; [6]Center for Fetal Medicine and Prenatal Genetics, Department of Obstetrics and Gynecology, Brigham and Women's Hospital-Harvard Medical School, Boston, MA.*
Objective: Galectins are proteins with a regulatory role in the immune response. Members of this family play a role in pregnancy complications. Galectins on human Chr.19 have been implicated in the immunology of placentation. Objectives of this study were to:1) characterize the expression pattern of the galectins; 2) investigate the evolutionary "birth and death process"of galectins.
Methods: qRT-PCR was performed on all galectins in placentas and fetal membranes. The groups studied were: a) human term not in labor (TNL, n=8); b) term in labor (n=9); c) baboon TNL (n=3). Differential expression was inferred using false discovery rate corrected p-values. Expression was localized by *in situ* hybridization and immunohistochemistry. DNA sequences were generated from human and other primates. Genome sequences from mammals were interrogated to define the evolutionary history of the cluster in mammals.
Results: 1) Galectins displayed an expression pattern which varied between the placenta and membranes. Eleven were predominantly expressed in villous tissue, and 5 in membrane; 2) Two galectins were differentially expressed during spontaneous labor in humans ($p<0.05$); 3) Two gene clusters (*LGALS4, LGALS7)* and (*LGALS13*, *sPP13*, *sCLC*, *LGALS14, CLC)* are on human Chr.19q13.2. The latter had higher expression in placenta than in membranes ($p<0.001$;16-2061 fold); 4) The 1st cluster is present in all mammals. The 2nd emerged recently and is present in primates. This cluster has been disrupted on particular lineages; *LGALS14* was excised during chimpanzee evolution. Splice variants were found for *LGALS13* that have not been seen in humans.
Conclusion: 1) Galectins show a pattern of differential expression measured by differences in transcript amount and splice variants; 2) The clusters on Chr.19 are characterized by rapid gene gain and loss; 3) These findings suggest a lineage- and tissue-specific expression profile.

810

Sequence Analysis of the Follicle Stimulating Hormone Receptor (FSHR) Using Long-Range Single-Stranded Conformation Polymorphism (LR-SSCP): A New, Highly Efficient, Non-Radioactive Mutation Detection Method. Maria N Thanasoula, Maria D Lalioti. (SPON: Emre Utku Seli). *Ob/Gyn and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: Single-stranded conformation polymorphism (SSCP) is a sequence variation detection method. Double-stranded PCR products are denatured to single-strands using formamide and heat, and allowed to obtain a unique conformation during electrophoresis in a non-denaturing gel. Traditionally, SSCP is performed on radioactive PCR products, less than 200 bp and run on a long acrylamide gel. In order to perform mutation analysis of the FSHR gene with a higher throughput and accuracy, without the need for extensive sequencing, we aimed to develop a more sensitive SSCP method that could be performed on larger, non-radioactive amplicons, run on a mini-gel.

Methods: We selected 3 different genes for which known mutations or polymorphisms have been described and were available in our lab. For each gene we designed 4 overlapping PCR amplicons that contained the sequence variation and whose length was between 153 and 756 bp. RNA and genomic DNA were isolated from human cumulus cells and mouse ovaries (Trizol). The human FSHR and the mouse Wnk4 and Epab genes were amplified by genomic PCR and RT-PCR, respectively. The PCR products were diluted with formamide-loading buffer and denatured at 95 °C for 10 min. They were electrophoresed in a non-denaturing 8x10 cm mini-gel (Biorad) at 250 V, for 4 hours, at 4°C. They were stained with SybrGold (Molecular Probes) and visualized.

Results: In order to exclude sequence-specific results, we tested 3 different genes: human FSHR and mouse Wnk4 and Epab genes. The mutations detected were 1 or 2 bp mismatches. We optimized the SSCP conditions to allow detection of a single base pair difference in large fragments (756 bp). The same results were obtained using regular or SSCP-specific acrylamide solutions. The detection efficiency of the method was 86 % (6/7 mutations tested). To validate the method, it was used to genotype the common p370Ala/Thr variant on FSHR from 80 patients. The FSHR was sequenced in 40 patients and confirmed the accuracy of the SSCP results.

Conclusions: LR-SSCP is a low-cost, highly efficient mutation detection method that can be applied to large PCR or RT-PCR fragments. It is performed and visualized on common laboratory equipment. Moreover, it can substitute sequencing and/or enzyme digestion for genotyping of known sequence variations on human or animal models.

811

Progesterone Induces Activation of Signal Transducer and Activator of Transcription 3 (STAT-3) by Serine Phosphorylation at Ser 727 in Human Endometrial Epithelial Cells (HEECs). Shumei Zhao, Chainarong Choksuchat, Todd D Deutch, Thomas D Kimble, David F Archer.* *Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA, USA.*

Objectives: Endometrial bleeding is common problem in women taking progestin-only contraception, the mechanisms are poorly understood. Signal transducers and activators of transcription (STATs) are a family of transcription factors which were originally identified on the basis of their ability to transduce a signal from a cellular receptor into the nucleus and modulate the transcription of specific genes. It is not well-known about progesterone-mediated signaling pathways in human endometrial epithelia cells. The objective of this study is to investigate the progesterone signal transduction pathways in order to elucidate the potential mechanisms involved in progesterone-induced uterine bleeding. **Material and Methods:** Western immunoblotting was used to analyze the expression, activation and regulation of STAT-1, STAT-3, STAT-5b, as well as Suppressor of Cytokine Signaling (SOCS)-3 after addition of progesterone with or without antiprogestin (RU486) to HEECs at various time points. The phosphorylation of STAT-3 and STAT-5b was evaluated further by immunofluorescenc analyses. **Result:** We found an elevated expression of STAT-3 and the phosphorylation of STAT-3 at Ser727, but not Tyr705, within 30 minutes following progesterone treatment by Western immunoblotting analyses. Maximal activation of STAT 3 was observed in 60 minutes, but decline by 48 hours. Maximum translocation of STAT 3 proteins from the cytoplasm to the nucleus was also observed in 60 minutes incubation with progesterone by immunofluorescence analyses. STAT-3 transcriptional activation by progesterone in HEECs was not inhibited by RU486. Progesterone treatment induced STAT-5b but not activation. There was no STAT-1 expression or activation detected in this study. Suppressor of cytokine signaling (SOCS-3)-3 was detected upon stimulation with progesterone. **Conclusion:** These data demonstrate a strong association of progesterone induces STAT3 activation by serine phosphorylation at Ser 727, and STAT-3 activation may play an important role in progesterone-induced uterine bleeding.

812

NANOS3 Mutation Analysis in 80 Chinese Women with Premature Ovarian Failure. Yingying Qin,[1,2] Han Zhao,[1,2] Ertug Kovanci,[2] Joe Leigh Simpson,[*2,3] Zijiang Chen,[1] Aleksandar Rajkovic.[*2] *[1]Reproductive Medical Center, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China; [2]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.*

OBJECTIVE: *NANOS3,* encoding an RNA binding protein, was first identified as a maternal effect gene in Drosophila and has a conserved function in germ cell development. Absence of *Nanos3* results in both male and female infertility in mice. Our objective was to investigate whether mutations in *NANOS3* exons were present in Han Chinese women with premature ovarian failure (POF).

DESIGN: Mutation screening of prospectively collected DNA samples.

MATERIALS AND METHODS: Our study population comprised eighty Han Chinese women with POF ascertained at Shandong Provincial Hospital of China. Recruitment criteria were defined as menopause occurring before 40 years and at least twice serum follicle stimulating hormone (FSH) concentrations that exceeded 20 IU/ml. Women with chromosomal abnormalities were excluded. Mutation screening for two exons was performed using polymerase chain reaction (PCR) with 3 pairs of specific primers and denaturing high-performance liquid chromatography (DHPLC) on the WAVE System 3500 (Transgenomic Ltd, Omaha, NE) followed by sequencing on an automated sequencer, ABI PRISM 310 (Applied Biosystems, Foster City, CA) when heteroduplexs were detected. Further studies of variation utilized an optimized PCR-RFLP protocol.

RESULTS: None of the eighty POF subjects showed mutations in the *NANOS3* gene. A single known synonymous SNP (c. 356A>G) in exon 1 was identified by sequencing. This SNP was found in 45 POF as 34 heterozygotes and 11 homozygotes using RFLP with MluI restriction enzyme digestion. No additional SNPs or mutations were identified in the coding exons of *NANOS3*.

CONCLUSION: Mutations in *NANOS3* exons are rare in Han Chinese subjects with POF.

813

Progesterone Induced Mitochondrial Hyperpolarization in MCF-10A Breast Epithelial Cells. Millie A Behera, Nikki Saner, Thomas M Price.* *Obstetrics and Gynecology, Duke University, Durham, NC, USA.*

Traditionally, the reproductive hormone progesterone regulates gene transcription via typical nuclear steroid receptors. Yet, other actions of progesterone are rapid and occur via poorly described non-genomic mechanisms. We have previous identified a truncated progesterone receptor, PR-M, localized to the mitochondria by techniques including Western blot analysis after cellular fractionation, immunofluorescent localization of a GFP tagged recombinant PR-M and Western blot analysis of purified human heart mitochondrial proteins. **OBJECTIVE:** The purpose of this study was to demonstrate a possible action of progesterone on the mitochondria using a cell line known to express PR-M but lack expression of the known nuclear progesterone receptors. **METHODS**: Using the MCF-10A breast epithelial cell line, changes in mitochondrial membrane potential were determined with the mitofluor, JC-1. JC-1 is a cationic dye that exhibits membrane potential dependent accumulation in the mitochondria with a fluorescence emission shift from green (~529nm) to red (~585nm). Mitochondrial depolarization correlates with a decrease in the red/green emission ratio, whereas hyperpolarization would be shown by an increase. Assays were performed in 48-well plates using a fluorescent plate reader. **RESULTS:** Reliability of the JC-1 assay was first determined by induction of mitochondrial membrane depolarization with the calcium ionophore, A23187. Preliminary assays determined the appropriate number of cells to yield a maximal result at 50K per well. Subsequent studies revealed a dose dependent induction of mitochondrial hyperpolarization with a 30-60 min treatment with 10^{-6} to 10^{-8} M progesterone or the synthetic progestin R5020. The reaction was inhibited by pretreatment with the specific PR antagonist, RTI 6413-049b. **CONCLUSIONS:** Using a breast epithelial cell line known to lack nuclear PR, but express mitochondrial PR, we have shown an effect of progesterone on increasing mitochondrial membrane potential. Mitochondrial membrane hyperpolarization may have several causes, one of which is a higher proton pumping activity by respiratory chain enzymes. This brings forth the hypothesis that the well-known progesterone associated increase in metabolic rate may be due to a direct action on mitochondria.

814

GDF9 Mutation Analyses in 100 Chinese Women with Premature Ovarian Failure. Han Zhao,[1,2] Yingying Qin,[1,2] Ertug Kovanci,[2] Joe Leigh Simpson,[*2,3] Aleksandar Rajkovic,[*2] Zijiang Chen.[1] *[1]Reproductive Medical Center, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China; [2]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.*

OBJECTIVE: Growth differentiation factor 9 (GDF9) is a germ cell specific growth factor secreted by oocytes and crucial for folliculogenesis. Our goal was to determine if perturbations in the GDF9 gene occur in Chinese women having Premature Ovarian Failure (POF).

DESIGN: Mutation screening of prospectively collected DNA samples.

MATERIALS AND METHODS: 100 POF women and 96 control women, were recruited in the Reproductive Medical Center, Shandong Provincial Hospital, China. Inclusion criteria were defined as twice serum follicle stimulating hormone concentrations greater than 20IU/ml before age of 40 years, and normal karyotype. After genomic DNA was extracted from blood samples, the coding regions of GDF9 were amplified using polymerase chain reaction (PCR) with 3 pairs primers. Heteroduplexes were detected using denaturing high-performance liquid chromatography(DHPLC). Samples which demonstrated heteroduplex formation on DHPLC were then sequenced directly after PCR amplification on an automated sequencer.

RESULTS: In the POF group, we found 3 nonsynonymous SNPs in the GDF9 gene: c.436C>T (p.Arg146Cys), c.712A>G (p.Thr238Ala) and c.1283G>C (p.Ser428Thr). Novel mutations c.436C>T and c.1283G>C were also discovered in the controls. The only nonsynonymous SNP (c.712A>G) detected in POF group but not in controls was present in a 30-year-old woman with secondary amenorrhea. In addition, we found 3 synonymous (silent) mutations in the POF group, two also present in controls.

CONCLUSION: Although 4 novel SNPs and 2 additional known mutations were found in the POF cases, all except one (c.712A>G) was either found in controls or was silent. 712A>G mutation was not present in controls. 712A>G mutation in GDF9 may be associated with POF in our study population.

815

In Vitro Metabolism of 17α-Hydroxyprogesterone Caproate (17-HPC) by Hepatocyte Suspensions. Richard H Lee,[1] Frank Z Stanczyk,[*1] Thomas M Goodwin,[*1] Durlin E Hickok.[2] *[1]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA; [2]Adeza Biomedical, Sunnyvale, CA, USA.*

Introduction: 17-HPC has been shown to significantly reduce the rate of recurrent preterm birth among women at high risk for preterm birth. However, little is known about its metabolism. The objective of the present study was to gain insight into the metabolism of 17-HPC by determining which metabolites are formed during incubation of 17-HPC with human hepatocytes *in vitro*.

Methods: Concentrations of 2 and 20 μM 17HPC were incubated in duplicate at 37°C for 4 hours with human hepatocytes in an atmosphere of 95% O2/ 5% CO2. As a positive control, hepatocytes were incubated with 7-hydroxycoumarin. Other controls included hepatocytes without 17-HPC and 17-HPC without hepatocytes. The reactions were stopped with acetonitrile, and the products were isolated by HPLC and identified by LC-MS.

Results: Twenty metabolites of 17-HPC were detected; 11 metabolites were present in both the 2 and 20 μM 17HPC incubations, of which most were tentatively identified as acetylated conjugates, some were sulfates, and the rest were unconjugated. No loss of the caproate group was found. Further identification of four major metabolites showed conjugation with acetate, hydroxylation, and formation of double bonds. Metabolites of 7-hydroxycoumarin included sulfates and glucuronides but not acetates.

Conclusions: 1) Unlike progesterone and 17α-hydroxyprogesterone, which are conjugated primarily as glucuronides, 17HPC undergoes extensive acetylation; 2) no loss of the caproate group was found, which is consistent with the limited published *in vivo* findings.

816

Characterizing the Regulation of 17-20,Lyase Activity of Marmoset and Rhesus P450c17 cDNAs Expressed in HEK-293 Cells. J Christina Pattison,[1] Ann D Nguyen,[2] Alan J Conley,[2] Ian M Bird.[*1] *[1]Perinatal Research Labs, Dept OB/GYN, Univ of Wisc-Madison, Madison, WI, USA; [2]Population Health and Reproduction, Univ of Cali-Davis, Davis, CA, USA.*

We have previously shown that 17,20-lyase activity of cytochrome P450c17 is positively correlated with cytochrome b5 (cytb5) expression in marmoset monkeys by *in vivo* and *in vitro* methods. Circulating DHEA levels and cytb5 expression are lower in marmosets than rhesus. We have also shown that the marmoset P450c17 amino acid sequence is 82.4% and 85% homologous to the human and rhesus sequences, respectively. Our objective in this study was to confirm the role of cytb5 on 17,20-lyase activity, as well as investigate the importance of a specific amino acid difference between the marmoset and rhesus/human sequences. Specifically, multiple sequence alignments, along with prediction of putative PKA and PKC phosphorylation sites, led us to investigate the role of Valine 344 in the marmoset compared to Isoleucine in the rhesus/human. Herein, we provide evidence for cytb5 regulation of 17,20-lyase activity in both monkey microsomes and P450c17 cDNAs transfected in HEK-293 cells. Addition of recombinant cytb5 to marmoset adrenal microsomes resulted in increased 17,20-lyase activity. Co-transfection of rat cytb5 cDNA with marmoset and rhesus wild-type P450c17 cDNAs, as well as the marmoset V344I mutant cDNA, increased DHEA production by all plasmids. While we also measured the effect of kinase agonists, such as Forskolin and TPA, on DHEA production in transfected HEK-293 cells, the most profound effect was from co-expression of cytb5. Despite the difference in the magnitude of 17,20-lyase activity between marmosest and rhesus P450c17 cDNAs, cytb5 had a similar, significant effect. Changing the Valine 344 in the marmoset P450c17 sequence to the Isoleucine 344 in rhesus/human did not change the scale of 17,20-lyase activity in the marmoset mutant to approximate the rhesus wild-type. These combined results confirm that cytb5 level is likely the primary regulator of 17,20-lyase activity in marmosets in vivo. Although specific amino acid differences between species may be a factor in 17,20-lyase activity, the V344I difference between marmoset and rhesus/human is not responsible for the observed differences in 17,20-lyase activity between these species. Further studies are necessary to elucidate the importance of other amino acid differences between sequences along with post-translational phosphorylation events.

817

Fetal Colonic Enteric Nervous System Is a Site of Glucocorticoid-Induced Gastrointestinal Maturation. Jayaraman Lakshmanan, Guong L Liu, Gyu Y Choi, Noboru Oyachi, Michael G Ross.[*] *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Despite the diversity of fetal glucocorticoid (GC) maturational effects, there is little understanding the developing enteric nervous system (ENS) response to GC. Administration of betamethasone (a synthetic GC) to the preterm ovine fetus greatly augments cholinergic-mediated colon contractility. Based on this finding, we hypothesize that the developing ENS, similar to the central nervous cholinergic system, is a target for GC action. We sought to confirm our hypothesis by examining the ontogenic profile of plasma cortisol and GC-receptor (GCR) density in the distal colon ENS of ovine fetuses during the last 25% of gestation.

Method: Plasma samples and intestinal segments (20-25 cm) were obtained from uninstrumented ovine fetuses sacrificed at very preterm (VPT: 118-120 days), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (T: 146-147 days). Fetal distal colonic segments were fixed in Bouin's solution, and paraffin sections immunostained with glucocorticoid receptor antibody (sc8992, Santa Cruz). Immunostaining was performed by avidin-biotin-peroxidase system using Vectastain ABC-kit (Vector Laboratories, Inc). Cortisol levels were quantified using commercial radioimmunoassay kit. The number of myenteric and submucosal ganglionic neurons positive for GCR antibody staining were counted under microscope. Statistical differences between different groups were analyzed by one-way ANOVA.

Results: Plasma cortisol levels (700±65 pg/ml) were similar in VPT and PT fetuses, increased in NT fetuses (2,000±135 pg/ml) and markedly increased in T fetuses (25,400±1250 pg/ml). The percent of myenteric and submucosal neurons positive for GCR immunostaining was maximal at near term gestation with a statistically significant and dramatic reduction at term (myenteric VPT: 66.6±2.7, PT: 75.6±0.3, NT: 86.0±0.8 and T: 9.3±3.3%). (submucosal VPT: 32.4±4.0, PT: 41.5.6±3.7, NT: 75.0±2.5 and T: 12.6±2.0%).

Conclusion: A significant rise in plasma cortisol is first observed in near term fetal sheep, in conjunction with maximal positive GCR staining in the ENS neurons. The precipitous decrease in the number of neurons exhibiting GCR at term is likely due to downregulation mediated by high plasma cortisol. In view of the previously observed GC-induced augmentation of distal colon contractile responses, we conclude that the ENS is a target for GC-mediated intestinal maturation.

818

Effect of Dexamethasone on Markers of Neuronal Injury in the Newborn Guinea Pig. Endla K Anday,* Qazi M Ashraf, Kristie Hornick, Anli Zhu, Om P Mishra. *Pediatrics, St. Christopher's Hospital for Children, Philadelphia, PA, USA.*

Background: Glucocorticoids have been used to treat chronic lung disease in the prematurely born infant. However, there is an increased risk for adverse neurological outcome through mechanisms that are not entirely clear. Previously, we have shown that antenatal administration of Dexamethasone results in increased Ca^{++}-influx in neuronal nuclei of the guinea pig fetus.

Objective: The present study tests the hypothesis that postnatal Dexamethasone administration will result in increased neuronal nuclear Ca^{++}-influx in the newborn guinea pig, leading to activation of caspase -9 and -3 triggering the apoptotic pathway.

Design/Methods: 17 newborn guinea pigs <5 days of age were studied: Saline (**Sa**) n=6 or Dexamethasone, 0.4mg/kg/dose (**Dx**) n=11 was injected i.p. daily x 3 days. Brains were harvested at 72 hours post **Sa** or **Dx**. Nuclear Ca^{++}-influx, cytosolic Caspase-3 and -9 expression were determined. Proteins were separated and incubated with anti-Bax, anti-Bcl-2, anti-active caspase-3 and anti-active caspase-9, respectively. Protein bands were detected and analyzed. Caspase-3 and -9 activity was determined using specific fluorogenic substrates.

Results: Ca^{++}-influx (pmoles/mg pro) was 3.690.56 (**Sa**) and 5.841.03 (**Dx**), $P<0.01$. Bax expression (OD x mm^2)was 77.834.26 (**Sa**) and 154.6415.18 (**Dx**), $P<0.01$; Bcl-2 expression was 173.6025.23 (**Sa**) and 183.128.24 (**Dx**), P=NS. Caspase-9 expression was 35.811.50 (**Sa**) and 64.331.56 (**Dx**), $P<0.01$; Caspase-9 activity (nmoles/mg pro/hr) was 4.030.46 (Sa) and 5.210.75 (**Dx**), $P<0.05$. Caspase-3 expression was 96.2817.07 (**Sa**) and 133.2247.35 (**Dx**), $P<0.05$; Caspase-3 activity (nmoles/mg pro/ hr) was 20.052.85 (**Sa**) and 20.202.71 (**Dx**), P=NS. The data show that postnatal Dexamethasone results in increased Ca^{++}-influx in neuronal nuclei of newborn guinea pigs leading to increased expression of Bax, caspase-9 and -3 expression with activation of caspase-9.

Conclusions: We conclude that Dexamethasone administered postnatally activates neuronal nuclear membrane mechanisms that initiate the proapoptotic cascade. We speculate that Dexamethasone modifies nuclear membrane mechanisms of Ca^{++}-influx and modulates nuclear transcription factors resulting in an increased expression of proapoptotic genes relative to antiapoptotic genes that lead to neuronal injury. (Funded by NIH-HD 20337, 38079 and St. Christophers Foundation)

819

Cellular Localization of Corticotrophin Releasing Factor Binding Protein (CRF-BP) in Fetal Ovine Distal Colon: A Possible Local Inhibitor of Stress-Induced Colonic Motility. Jayaraman Lakshmanan, Guong L Liu, Noboru Oyachi, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: Stress induced increases in colonic motility and in utero meconium passage requires participation of key molecules of the CRF system in the contractile apparatus of colon. We have demonstrated immunohistochemical evidence for the expression of both CRF-receptors R1 and R2 in distal colonic segments of ovine fetuses. In the present study we sought to identify the cellular localization of CRF-BP, a protein known to compete with CRF-R1 and CRF-R2 for CRF, and thus inhibit the biological functions of CRF.

Method: Distal colon (20-25 cm in length) was dissected from ovine fetuses of different gestational ages (n=4 at each age): very preterm (VPT: 118-120 days), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (T: 146-147 days). Four cm segments were prepared and colonic rings (3-4 mm thickness) were made from both ends of each segment, fixed in Bouin's solution, paraffin embedded and sections subject to immunostaining with rabbit polyclonal antibody to CRF-BP (sc 20360, Santa Cruz). Immunoreactive materials on the sections were identified by avidin-biotin-peroxidase system using Vectastain ABC-kit (Vector Laboratories, Inc) and 3, 3'-diaminobenzidine (Sigma) as a chromogen. Percent of myenteric and submucosal ganglia expressing CRF-BP were counted microscopically and analyzed by ANOVA.

Results: The CRF-BP antibody immunostained smooth muscle layers, enteric ganglia and epithelial layers. The percent of myenteric ganglia (VPT: 42 ± 3, PT: 40 ± 3, NT: 43 ± 2 and T: 50 ± 4%) and submucosal ganglia (VPT: 37 ± 4, PT: 31 ± 5, NT: 32 ± 4 and T: 46 ± 5%) expressing CRF-BP protein remained nearly constant from very preterm through nearterm, with a significant ($p<0.05$) increase occurring at term.

Conclusion: We conclude that CRF-BP is locally expressed in ovine fetal colon throughout gestation. Marked increases observed at term indicate that neuronally expressed CRF-BP may compete with CRF for CRF-R1 and thus prevent stimulation of distal colonic motility. We speculate that delayed or inhibited CRF-BP expression may potentiate in utero MEC passage at term.

820

In Utero Resuscitation: Fetal Recovery from Umbilical Cord Occlusion (UCO) Induced Metabolic Acidosis. John D Richard, Jayaraman Lakshmanan, Guong L Liu, Reuben Lakshmanan, Jordan Ross, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objectives: Umbilical cord or immediate neonatal blood gas values have been utilized to determine the effect of labor-induced fetal heart rate changes on fetal acidosis. Worsening acidosis during the immediate neonatal period may be attributed to continued neonatal hypoxemia or hypoperfusion. Alternatively, it has been hypothesized that "washout" of metabolic acids from an *in utero* insult results in exacerbated measures of acidosis despite newborn clinical improvement. In the present study we sought to determine the rate of exacerbation or recovery of blood gas measures following cessation of repeated UCO resulting in severe acidosis.

Methods: Near-term ovine fetuses (N=6, 129±2 dGA) underwent chronic preparation of fetal brachial artery catheters and placement of an inflatable umbilical cord occluder. Following a minimum 3 d recovery from surgery, fetuses underwent a 2 to 4 h series of progressive 45-60 s UCO at 2-3 min intervals until fetal arterial pH decreased to 7.0. Fetal arterial blood samples were drawn at baseline, at 1-5 min following UCO at maximum acidosis, and at 1, 2 and 24 h recovery.

Results: At maximum acidosis, fetal base deficit (BD) averaged 16.3±2.7 mmol/l. With the cessation of UCO, fetal BD decreased to 5.6±3.8 within 2 h and to basal values (-5.0±2.6 mmol/l) at 24h. Within the first 2 h BD decreased at a rate of 5.4 mmol/h. There was no evidence of exacerbation of BD in the post UCO recovery period as studied in any fetus.

Conclusions: The results of the present study demonstrate that severe fetal acidosis resulting from repetitive UCO may resolve in utero at a maximum rate of 1 mmol/10 min, in the absence of uterine contractions or continued fetal compromise. There is no evidence of a fetal tissue metabolic acid "washout" during the resolution of asphyxia. These rates may provide a basis for for the timing of a prelabor fetal asphyxial event.

821

Evidence for Pre-Receptor Metabolism of Glucocorticoids in Ovine Fetal Distal Colonic Enteric Nervous System. Jayaraman Lakshmanan, Guong L Liu, Noboru Oyachi, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Objective: We recently demonstrated the presence of glucocorticoid receptors (GCR) in the enteric nervous system (ENS) of ovine fetal distal colon and hypothesized a pivotal role for GC in colonic contractile maturation and meconium passage. GC actions at the cellular levels are controlled by 11 beta-hydroxydehydrogenase (11β HSD) type I and type 2, enzymes which regulate the interconversion of active GCs and their inactive 11-keto metabolites. In the present investigation we sought to demonstrate the presence of 11β HSD-1 and HSD-2 in the fetal ENS as evidence that these enzymes provide a novel means for regulation of GC-mediated activities.

Method: Bouin's solution fixed, paraffin sections of distal colonic segments were prepared from ovine fetuses (n=4 at each age) at very preterm (VPT: 118-120 days), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (T: 146-147 days). Sections were immunostained with antibodies to 11β HSD-1 and 11β HSD-2 (sc: 20715, sc:20716 Santa Cruz) by avidin-biotin-peroxidase system using Vectastain ABC-kit (Vector Laboratories, Inc) and 3, 3'-diaminobenzidine (Sigma) as a chromogen. The percent of ganglia positive for 11β HSD-1 and HSD-2 were counted microscopically. Statistical differences between age groups were analyzed by one-way ANOVA.

Results: The antibodies to 11β HSD-1 and HSD-2 strongly immunostained myenteric and submucosal neurons at all gestational ages. The percent of myenteric ganglia expressing 11β HSD-1 (VPT: 47.8±5.0, PT: 36.0.6±6.0, NT: 41.0±4.0 and T: 55.0± 6.0%) and HSD-2 (VPT: 53.0±5.0, PT: 44.0.6±7.0,

NT: 43.±4.0 and T: 64.0±7.0%) were significantly higher at term as compared to younger gestational ages. The percent of submucosal ganglia expressing 11β HSD-1 (VPT: 20.0±5.0, PT: 18.6±3.0, NT: 18.0±4.0 and T: 54.0±6.0%) at term markedly ($p<0.05$) increased while the expression of HSD-2 (VPT: 25.0±4.0, PT: 26.0±5.0, NT: 22.0±4.0 and T: 28±3.0%) remained constant at all gestational ages.

Conclusion: Isoenzymes of 11β HSD-1 and HSD-2 are expressed both in myenteric and submucosal neurons. We speculate that increasing levels of 11β HSD-1 near term contributes to glucocorticoid-induced gastrointestinal maturation.

822

Identification of High and Low-Molecular Weight Corticotrophin Releasing Factor Receptor in Rat and Sheep Fetal Intestine. Jayaraman Lakshmanan, John D Richard, Theresa A John, Reuben Lakshmanan, Jordan Ross, Michael G Ross.* *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr, Torrance, CA, USA.*

Hypothesis: Corticotrophin releasing factor (CRF) mediates its biological effects by two different CRF receptors (CRF-R1 and CRF-R2). Cloned CRF receptor cDNA structure predicts that both receptor proteins are of similar molecular size (40-48kDa) and contain multiple potential sites for glycosylation. Both receptor mRNAs were also shown to undergo splicing, predicting that receptors may exist in multiple variant forms. We recently reported hypoxia-induced increased plasma CRF and in utero meconium passage in fetal rats, and hypothesized that hypoxic stress-induced colonic motility is mediated by CRF. In support of our hypothesis we recently immunolocalized CRF-receptors in rat fetal gastrointestinal (GI) tract and ovine fetal distal colon. Here, we sought to identify the molecular nature of CRF-receptors by Western blot analysis with sensitive chemiluminescent detection.

Method: Whole GI tracts of rat fetuses (n=11) at e19 (term=22 d) and distal colonic segments of ovine fetuses (n=6) at 147 d (term=148-152 d) were homogenized in membrane extraction buffer (10mM $NaPO_4$, pH7.4, 1%NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 200μM sodium orthovanadate and protease inhibitors) and the extracts subjected to Western blot analysis with antibody to CRF-R1/R2 receptors (Santa Cruz Biotechnology) or to CRF-R2 (Santa Cruz Biotechnology).

Results: The rabbit polyclonal CRF-R1/R2 receptor antibody identified 235, 64, 44 and 20 kDa immunoreactive proteins both in fetal rat GI tract and sheep distal colon. The CRF-R2 antibody identified a major 80 kDa protein and two minor 26 and 20 kDa proteins in rat fetal GI tract.

Conclusion: The identification of CRF-receptor immunoreactive proteins with molecular weights (235, 64, 80 kDa) greater than the size (40-48kDa) predicted by the cloned CRF-R cDNAs suggests that CRF receptors undergo site specific glycosylation. The findings of 44 and 20 kDa proteins suggest that glycosylated CRF-Receptors undergo deglycosylation or proteolysis during homogenization. The presence of 26 and 20 kDa immunoreactive proteins to CRF-R2 antibody is intriguing since these sizes correspond to recently discovered truncated CRF-R2 receptor. In summary, both fetal rat GI tract and fetal sheep distal colon contain glycosylated full length CRF-R1 and CRF-R2 receptors in addition to truncated CRF-R2 receptor.

823

SLC35C2 Subcellular Localization and Regulation of Sialyl Lewis X Antigen. Steve Dang,[1] Jennifer Belna,[1] Stephen Krawetz,[2] Richard Leach.*[1] *[1]Department of Obstetrics and Gynecology, University of Illinios, Chicago, IL; [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI.*

Objective: SLC35C2 (alias:OVCOV1) homologous to FUC1, a fucose transporter, is expressed in an immortalized human cytotrophoblast cell line, HTR-8/SVneo (HTR) under hypoxic conditions (Mammalian Genome 13, 619–24, 2002). The highly fucosylated sialyl Lewis X antigen (SLe^x) binding of selectin initiates cell adhesion to vascular endothelial cells identified both in inflammation and cancer metastasis. The objective of the present study was to determine SLC35C2 subcellualr localization and determine its role in SLe^x accumulation in HTR cells.

Results: RT-PCR demonstrated two SLC35C2 gene isoforms expressed in human HTR cells as predicted by NCBI (Accession Numbers; NM_015945 and NM_173073). Western blot demonstrated a predominant and specific protein band at 50 kDa. Both gene and protein expression is upregulated at 2% compared to 10% O_2. SLC35C2 protein co localized to the Golgi apparatus by confocal microscopy, similar to its homologue FUC1. Both gene and protein expression is inhibited by short interference RNA (siRNA) transfection (10nM and 100nM). SLe^x expression on HTR cells determined by flow cytometry is increased under 2% conditions and significantly reduced with treatment of 10 nM siRNA for 72 hr.

Conclusion: SLC35C2 originally isolated from the ectoplacental cone from a 7.5 dpc mouse implantation site plays an important role in the accumulation of SLe^x on human trophoblast cells. The early attachment trophoblast cells to the underlying maternal stroma and spiral artery endothelium are necessary for successful implantation to occur. These findings implicate SLC35C2 as an important mediator of this process through its role in SLe^x regulation.

824

The Cost-Effectiveness of Universal Screening for Subclinical Hypothyroidism. Stephen F Thung,[1] Edmund F Funai,*[1] William A Grobman.[2] *[1]Department of Obstetrics and Gynecology, Yale University, New Haven, CT, USA; [2]Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Objective: To determine if, and under what circumstances, routine screening for subclinical hypothyroidism (SH) during pregnancy would be cost-effective.

Methods: We developed a decision analysis model to compare the cost-effectiveness of two screening strategies during pregnancy for SH: (1) no routine screening of serum TSH levels (standard of care) and (2) routine screening of first trimester TSH levels. In the second strategy, a TSH above the 97.5th percentile was followed by a reflex free T4 to exclude overt hypothyroidism, and women diagnosed with SH received thyroid replacement. We assumed, based on observational studies, that thyroid hormone replacement could reduce the incidence of an IQ < 85 in offspring of women with SH by 2/3, although this value was varied widely in sensitivity analysis. Other key assumptions were: the costs of screening ($25), surveillance and therapy ($100), and the discounted cost of additional care/education for offspring with an IQ 70 - 85 ($27,500) and with an IQ < 70 ($315,000). Offsprings' QALY's were calculated from annual utility rates based upon their IQ: IQ > 85 = 1.0, IQ 70-85 = 0.9 and IQ < 70 = 0.8. These assumptions were also varied widely in the sensitivity analysis. The main outcome measure was marginal cost per QALY.

Results: Our model predicts that, under baseline assumptions, screening is both cost saving and more effective than the present standard of care. Specifically, for every 100,000 women screened, screening saves $10,360,000 and adds 677 QALY's as compared to routine care. In sensitivity analysis, these results proved to be robust to wide changes in variable estimates. Screening remained cost-effective (<$50,000/QALY) unless thyroid hormone replacement failed to reduce the chance of an IQ<85 by at least 4%.

Conclusion: Screening for SH in pregnancy will be a cost-effective strategy under a wide range of circumstances. A randomized controlled trial to evaluate the efficacy of thyroid hormone replacement in SH women is needed.

825

The MFMU Cesarean Registry: Why Is the VBAC Rate Falling? William A Grobman. (SPON: Michael L Socol). *For the NICHD MFMU Network, Bethesda, MD, USA.*

Objective: The vaginal birth after cesarean (VBAC) rate has plummeted in the US over the past decade. It remains unknown if this is due to (1) preferential exclusion of the candidates most likely to fail a trial of labor (TOL), (2) a more widespread and non-selective reduction in TOL even among women who are highly likely to have a VBAC, or (3) a greater likelihood to abandon a TOL once it has been undertaken. The objective of this study was to determine the management change that has been primarily responsible for the changing VBAC rate.

Methods: All women eligible for a TOL with one prior low transverse cesarean and a vertex singleton gestation were identified in a concurrently collected database of deliveries occurring at 8 academic centers during a four-year period (1999 – 2002). Using a validated model for prediction of successful VBAC for women undergoing a TOL (Am J Obstet Gynecol 2005;193:S125) patients were classified by their predicted chance of vaginal delivery. Four-year trends analyzed included: overall VBAC rate (vaginal birth/women eligible for TOL), the proportion of women who attempted a TOL stratified by decile of predicted chance of VBAC, and the proportion of women who achieved a vaginal birth after a TOL stratified by decile of predicted chance of VBAC.

Results: 14,276 women met criteria for analysis. From 1999 to 2002, the VBAC rate underwent a steady decline: 55% to 50% to 41% to 35%, $P < .001$. The frequency with which women attempted a TOL progressively decreased during the four-year period regardless of a woman's predicted chance of vaginal

delivery (see table, test of trend P < .001 for each probability group). Conversely, there was no consistent evidence of any trend in likelihood that, once a TOL was attempted, successful vaginal delivery occurred (data not shown).

Conclusions: The steady decline in the VBAC rate is primarily due to the decrease in the frequency with which women attempt a TOL, and this decrease is evident among all women regardless of their predicted chance of a vaginal birth.

% ELIGIBLE WOMEN ATTEMPTING TOL STRATIFIED BY YEAR AND PREDICTED CHANCE OF VAGINAL DELIVERY

	≤ 40%	41 - 50%	51 - 60%	61- 70%	71 - 80%	81 - 90%	> 90%
1999	45%	56%	56%	53%	70%	82%	96%
2000	36%	48%	49%	47%	60%	73%	93%
2001	33%	31%	35%	39%	47%	68%	86%
2002	14%	28%	30%	33%	37%	58%	83%

826

Author Evaluation of Editorial Review. Mark Gibson,[*1] James R Scott,[*1] Catherine Y Spong.[*2] [1]*Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA;* [2]*Pregnancy and Perinatology Branch, NICHD, NIH, Bethesda, MD, USA.*

Objective

To evaluate authors' perceptions of editorial review for *Obstetrics & Gynecology*

Methods

Authors of 436 research manuscripts (May - December, 2005) were invited to assess editorial review quality in six domains (accurate, informed, impartial, important, respectful and constructive) and submission processes in three domains (ease, turnaround time, staff support), using a five point Likert scale. Overall satisfaction and likelihood of future submissions was assessed using yes/no responses. Each submitted article was reviewed by a journal editorial board member and two or three ad hoc expert reviewers; study respondents evaluated each reviewer separately.

Results

The response rate was 49%. Response rate did not differ by country of origin, but authors of accepted articles were twice as likely to complete the survey as authors of rejected manuscripts. (92 of 122 vs. 121 of 314). Although 88% of respondents expressed overall satisfaction with the review process, this differed according to whether the article was accepted or rejected (98% vs. 80%, p < 0.001). Respondents were more likely to rate procedures and processes for review positively than editorial commentary (88% vs. 69%, p<0.001). Among the six questions regarding content of editorial review, accuracy and respectfulness received the highest percentage of favorable ratings (each, 73%) while importance of editorial comments received the lowest percentage of favorable ratings (63%). For all aspects of content of editorial review, authors whose manuscripts were rejected gave favorable ratings 30% less often than authors whose articles were accepted (all, p< 0.001), and the lowest percentage of favorable ratings by authors whose manuscripts were rejected was given for importance of reviewers' comments (54%). Non-US respondents gave more positive assessments than US respondents, particularly with respect to positive ratings for the degree to which reviews were informative (71% vs. 63%, p<0.05) and constructive (72% vs. 64%, p<0.05). Ratings for ad hoc experts and the editorial board reviewers were similar. The percentage of favorable ratings for editorial board members (average number of reviews = 13) ranged from 51% to 85%.

Conclusion

Authors are most concerned that editorial reviews address issues they regard as important. Systematic collection of author feedback would assist medical journals in evaluation of editorial processes and review quality.

827

Relationship between Maternal Body Mass Index (BMI) and Post-Dates Pregnancy. Fiona C Denison,[1] Jackie Price,[2] Sarah Wild,[2] Cat Graham,[2] William A Liston.[1] (SPON: Hilary OD Critchley). [1]*Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, United Kingdom;* [2]*Publich Health Sciences, University of Edinburgh, Edinburgh, United Kingdom.*

Objective: To determine relationship between BMI and post-dates pregnancy.

Methods: A dataset containing all singleton births (n=186,087) in Sweden from 1998-2002 was obtained from the Swedish Medical Birth Register. Maternal variables included age, smoking status, ante and postnatal outcome, delivery gestation, height and weight during first trimester and prior to delivery (Body mass index (BMI) =weight (kg)/ height m^2). Infant characteristics included sex, live/stillborn, weight and length (Ponderal Index=birthweight (kg)/length $(m)^3$). Data were analysed by two-sample t-tests, linear regression modelling and ANOVA with multivariate analysis.

Results: After exclusion of records with missing or implausible data, 143,519 records were used for analysis. 7.8% of pregnancies delivered post-dates. Increasing maternal BMI, age and height at booking (10-12 weeks) were weakly associated with longer gestation (Pearson Correlation Coefficients: 0.05, 0.06 and 0.08, respectively; p<0.0001) with smoking being associated with shorter gestation (Pearson Correlation Coefficient -0.02; p<0.0001). A greater change in BMI (ΔBMI, difference between BMI at booking and delivery), resulted in a longer mean gestation (ΔBMI<0 kg/m^2, 281days;ΔBMI ≥10 kg/m^2 283 days; p<0.0001). However, BMI, age, height and smoking together accounted for less than 1.4% of the total variation in length of gestation in this population. Compared with a BMI of 20-25kg/m^3, increasing BMI was associated with a lower chance of spontaneous onset of labour (BMI 25<30kg/m^2 OR 0.71, 95% CI 0.69-0.73; BMI 30<35 kg/m^2 OR 0.57, 95% CI 0.54-0.60; BMI>35 kg/m^2 OR 0.43, 95% CI 0.4-0.47). In addition, compared to a BMI of 20-25kg/m^3 women with a BMI≥35kg/m^3 were significantly more likely to have a stillbirth (OR 3.89, 95% CI 2.44-6.22), pre-existing hypertension (OR 9.1; 95% CI 6.45-12.75), pregnancy induced hypertension (OR 4.24 95% CI 3.81-4.72), gestational diabetes (OR 5.6 95%CI4.61-6.83) and post-partum haemorrhage (OR1.34 95%CI1.16-1.55). BMI at booking and delivery correlated with ponderal index (r=0.06 and 0.12, respectively, both p<0.001).

Conclusion: Although increased maternal BMI (as well as age and height) correlate with longer gestation, much of the variation in gestation length remains unexplained. Women with a BMI≥35 merit increased surveillance due to obstetric risk.

828

Regional Poverty Rate Is a Risk Factor for Preterm Birth. Emily A DeFranco,[1] Louis J Muglia,[1] Mario Schootman.[2] (SPON: Yoel Sadovsky). [1]*Obstetrics and Gynecology, Pediatrics, Center for Preterm Birth Research;* [2]*Internal Medicine and Pediatrics, Washington University School of Medicine, St. Louis, MO.*

Objective: To determine if area-level poverty increases the risk of preterm birth over and above other individual risk factors.

Methods: We conducted a population-based cohort study to examine the association of regional demographic factors with preterm birth. Bivariate and multivariate logistic regression analyses assessed the association between county-level poverty within 115 counties in Missouri and preterm birth. We assessed the county-level poverty effect on preterm birth occurring at <37, <35, <32, and <28 weeks of gestation. We performed this analysis utilizing the Missouri Department of Health's database of births and fetal deaths from 1989 to 1997. Poverty rate was obtained from census data and was defined as the percentage of the population living below the US federal poverty line at the county level as a measure of area socioeconomic position. The poverty rate is a measure that is robust across various diseases and levels of geography; it has a link to possible policy implications, and is comparable over time.

Results: A total of 259,318 birth records were included in this analysis. We identified many individual factors associated with an increased risk of preterm birth. Those included non-white race, low income, low maternal or paternal educational level, recipient of state funded support programs (Medicaid, WIC, foodstamps), unmarried, inadequate prenatal care, medical complications, and tobacco or alcohol use. Women residing in counties with a high poverty rate (>20%) had in increased risk of preterm birth at each of the gestational age categories, even after adjustment for concomitant risks.

Conclusion: Women who reside in socioeconomically deprived areas are at increased risk of preterm birth, over and above other underlying risk factors. This emphasizes the importance of enhancing access to prenatal and preventative health care in areas with high poverty rates.

Effect of Regional Poverty Rate on Preterm Birth

Residence in High Poverty Area	OR	95% CI
All Preterm Births <37 weeks	1.28	1.17, 1.41
<35 weeks	1.34	1.18, 1.53
<32 weeks	1.54	1.29, 1.84
<28 weeks	1.63	1.23, 2.15

829

Paternal Race Is a Risk Factor for Preterm Birth. Emily DeFranco,[1,2] Lisanne Palomar,[2] Kirstin Lee,[2] Jenifer Allsworth,[1] Louis Muglia.[1,2] (SPON: Yoel Sadovsky). *[1]Obstetrics and Gynecology; [2]Pediatrics and Preterm Birth Research, Washington University School of Medicine, St. Louis, MO.*

Objective. There is a clear racial disparity in the occurrence of preterm birth, but little is known about the contribution of paternal race to this disparity. We tested the hypothesis that genetic factors, specifically paternal race, influence the risk for preterm birth.

Methods. We conducted a population-based cohort study to examine the association of paternal race with preterm birth. Multivariate logistic regression analysis was performed to examine this association in four parental race categories: white mother/ white father (W/W), white mother/ black father (W/B), black mother/ white father (B/W), and black mother/ black father (B/B). We performed this analysis utilizing the Missouri Department of Health's database of births and fetal deaths from 1989 to 1997.

Results. A total of 527,845 birth records were evaluated. The proportion of these births in the parental race categories was: 91.3% W/W, 1.1% W/B, 0.3% B/W, and 7.3% B/B. Compared to births to W/W parents, the risk of preterm birth at <35 weeks increased in pregnancies in which either parent was black. The highest risk was to pregnancies in which both parents were black, even when adjusting for other known risk factors for preterm birth (W/B, OR 1.31 [95% CI:1.14, 1.49], B/W OR 2.10 [95% CI: 1.68, 2.63], and B/B OR 2.27 [95% CI: 2.16, 2.38]). The OR for extreme preterm birth (<28 wks) was even higher among pregnancies with a non-white parent, W/B, OR 1.66 (95% CI: 1.25, 2.20), B/W, OR 2.58 (95% CI: 1.58, 4.21), and B/B, OR 3.64 (95% CI: 3.30, 4.00). The effect of paternal race remained a risk factor even when correcting for prior preterm birth: W/B, OR 1.89 (95% CI: 1.49, 2.57), B/W, OR 1.84 (95% CI: 0.94, 3.63), and B/B, OR 2.46 (95% CI: 2.21, 2.73).

Conclusions. This study demonstrates that the racial disparity in the occurrence of preterm birth is influenced not only by the mother's race, but also by the father's race. Paternal black race increases the risk of preterm birth in pregnancies to white mothers, suggesting a paternal genetic contribution to the fetal genotype which ultimately influences the risk for preterm delivery. Even after adjusting for other factors associated with preterm birth, paternal race exerts an even greater risk at the early extremes of gestational age when the prognosis is poorest. This data supports that both maternal and paternal genetic factors contribute to the timing of birth.

830

First vs. Second Trimester Dating Ultrasound: What Are the Differences in Outcomes? Aaron B Caughey,[1] James M Nicholson,[2] AE Washington.[1] (SPON: Linda C Giudice). *[1]Obstetrics, Gynecology, and Reproductive Sciences, UCSF, San Francisco, CA, USA; [2]Department of Family and Community Medicine, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: To examine the effect of obstetric ultrasound (OBUS) obtained in the first or second trimester on the measurement of the effect of complications ascribed to postterm pregnancies.

STUDY DESIGN: We conducted a retrospective cohort study of all term, singleton pregnancies delivered at our institution who had an OBUS at 24 weeks of gestation or less. Those women who underwent an OBUS at 12 weeks of gestation or less (OBUS12) were compared to those who had an OBUS at 13 to 24 weeks of gestation (OBUS13-24). The primary outcome measures were the rates of postterm pregnancies greater than 41 or 42 weeks' gestation. Secondary outcomes were the differences between the postterm and term gestations in maternal and neonatal outcomes.

RESULTS: In the OBUS12 group, the rate of postterm pregnancy > 42 weeks was lower (2.7%) as compared to the OBUS13-24 group (3.7%, p=0.022). With regards to reaching 41 weeks of gestation, the OBUS12 group was again lower (18.2%) as compared to the OBUS13-24 group (22.1%, p<0.001). There were also fewer postterm inductions at 42 weeks or beyond in the OBUS12 group (1.8%) as compared to the OBUS13-24 group (2.6%, p=0.017). When comparing perinatal outcomes between those women who reached 41 weeks of gestation and those prior to 41 weeks of gestation, the OBUS12 group demonstrated greater differences between these two groups.

CONCLUSION: Our findings suggest that earlier obstetric ultrasound, which leads to better pregnancy dating, reduces the rate of estimated postterm pregnancies. This may, in turn, reduce unnecessary intervention and lead to better identification of postterm pregnancies at greater risk of complications.

Comparing perinatal outcomes between gestations before and after 41 weeks

AOR (95% CI)	Mec	PPH	Bwt > 4,000
OBUS12	3.05 (2.40-3.87)	1.58 (1.12-2.24)	3.46 (2.94-4.08)
OBUS13-24	2.29 (2.00-2.62)	1.39 (1.14-1.70)	2.63 (1.94-3.54)

831

Documentation of GBS Status as a Metric of Racial/Ethnic Disparities in Obstetrical Care. Allison S Bryant, Yvonne W Cheng, Natali Aziz, Aaron B Caughey. (SPON: Julian T Parer). *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

OBJECTIVE:

Racial/ethnic disparities in processes of care have been demonstrated in many fields, but have not been extensively examined in obstetrics. We sought to determine whether race/ethnicity predicted documentation of Group B Streptococcus (GBS) status among women in the time between the releases of the 1996 and 2002 CDC guidelines regarding prevention of GBS.

STUDY DESIGN:

We conducted a retrospective cohort study of 8,199 women delivering at a single institution between 1996 and 2001. Women delivering at 37 weeks gestation or later were included. Bivariate analyses informed the creation of multivariate logistic models to predict documentation of GBS status. Of those women with documented GBS status, we then used the same models to predict GBS carriage.

RESULTS:

GBS status was documented for 27.3% of women in our study population. Significant predictors of GBS documentation, after adjustment for gestational age, number of prenatal care visits and year of delivery are presented in the Table. Of those women with documented GBS status, Black women had a higher adjusted odds of GBS carriage (AOR 1.52 [1.03, 2.22]).

CONCLUSION:

Despite having a higher risk of GBS carriage when screened, Black women were less likely to have their GBS status documented during the period of time when recommendations called for either screening- or risk factor-based approaches to GBS disease prevention. Asian women and those with public insurance were also less likely to be screened. This suggests that there are disparities in processes of care which may be related to differential health care settings or provider bias. Such process of care measures should be used more widely to document disparities in and quality of obstetrical care.

Adjusted odds ratios (AOR) for documentation of GBS status

Risk factor	AOR (95% CI)
Black race (vs. White)	0.74 (0.62, 0.89)
Asian race	0.75 (0.66, 0.85)
Hispanic ethnicity	0.92 (0.77, 1.10)
Maternal age >35	0.98 (0.86, 1.12)
Maternal age <20	1.00 (0.81, 1.24)
Public insurance	0.86 (0.74, 0.99)
UTI in pregnancy	1.34 (1.11, 1.64)
STI in pregnancy	1.00 (0.86, 1.16)

832

Predicting Small and Large for Gestational Age Fetus with First Trimester Maternal Serum fβhCG and PAPP-A. Daljit Sahota,[1] Karl Kagan,[2] Tze Lau,[1] Tak Leung,[1] Kypros Nicolaides.[2] (SPON: Carl Philip Weiner). *[1]Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong; [2]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, United Kingdom.*

OBJECTIVE: To determine whether fβhCG and PappA in maternal serum at the first-trimester screening for Trisomy 21 might detect women of high risk of having a small (SGA) or large for gestational age (LGA) birth.

METHOD: A retrospective analysis of 16,000 women having spontaneously conceived normal singleton term livebirth. Maternal age, height, weight, past obstetric history and ethnicity were recorded. Maternal fβhCG and Papp-A were measured using the Kryptor analyzer, converted to a multiple of their respective gestational specific median (MoM), corrected for maternal weight and ethnicity tehn transformed into the log domain. Birth weight (BW), gestational age and sex at delivery were recorded. BW and fβhCG and PappA MoMs were converted to their standard deviate equivalents ((value-expected)/sd), z-BW, z-$\log_{10}$fβhCG and z-Log_{10}PAPPA, where expected value for birth weight was the individualized birth weight adjusted for maternal age, height, weight, parity, gestational age and sex. Births were labeled as being SGA if z-BW was ≤-1.28, LGA if Z-BW ≥1.28 or appropriate for gestational age (AGA) if -1.28 < z-BW < 1.28. Z-PAPPA levels were similarly categorized as being either below Low (z-PAPPA ≤-1.28), High (z-PAPPA ≥1.28) or Normal (-1.28< z-PAPPA <1.28). Pearsons correlation coefficient was used to test for evidence of a linear relationship between z-BW and z-Log_{10}fβhCG and z-Log_{10} PAPPA. Mann-Whitney test was used to compare z-Log_{10}fβhCG and z-Log_{10} PAPPA between SGA, AGA and LGA births. Chi^2 test was used to test the association between fetal size and Low, High and Normal levels of PAPPA.

SATURDAY

RESULTS: z-Log_{10} PAPPA (r=0.19, p<0.0001 and z-Log_{10}fβhCG (r=0.06, p<0.0001) levels were significantly correlated with Z-BW. Low or high z-PAPPA levels were significantly associated with women who respectively delivered SGA babies or LGA babies. However, only 14% and 12% of women had Low or High PAPPA levels in the first trimester and an SGA or LGA baby.
CONCLUSION: Low PAPPA levels is associated with delivery of an SGA baby. High PAPPA is associated with delivery of an LGA baby. However, low or high PAPPA levels is not useful screening tool in the first trimester for predicting SGA or LGA fetuses once birth weight has been corrected for maternal and fetal characteristics.

833

Heterogeneity in the Epidemiology of Extremely Low Gestational Birth. Thomas F McElrath,[1] Olaf Dammann,[2] Elizabeth N Allred,[2] Alan Leviton.[2] (SPON: Robert L Barbieri). *[1]OB/GYN, Brigham & Women's, Boston, MA, USA; [2]Neuro Epidemiology, Children's Hospital, Boston, MA, USA.*
Objective: We describe the antecedents of delivery between 23 and 27 weeks in a prospectively collected cohort. We hypothesize that the contribution of specific antenatal complications to the incidence of preterm delivery will change with advancing gestational age.
Methods: Women at 14 tertiary centers underwent a standardized interview regarding pregnancy history in addition to a chart review. Each delivery was classified by complication: Preterm labor, preterm premature rupture of fetal membranes (pPROM), preeclampsia, placental abruption, cervical incompetence, and fetal distress/intrauterine growth restriction. These indications were examined for variation with gestational age and fetal number. Comparisons were made using T-test and one way ANOVA.
Results: 1,249 mothers were enrolled between 3/2002 - 8/2004. 19% of pregnancies were multiples. 13% of patients used fertility assistance. The incidence (%) of each complication was: Preterm labor (42), pPROM (22), preeclampsia (15), placental abruption (11), cervical insufficiency (5), and fetal indication/IUGR (4). Among singletons, the incidence of preterm labor significantly decreased (P<.01) while that of preeclampsia significantly increased (P<.01) with advancing gestational age. The incidence of the other complications did not vary. Among multiples none of the complications varied by gestational age. A significantly lower incidence of preeclampsia occurred among the multiples compared with the singletons (3 vs.18 percent; P<.01). The rate of complete steroid administration varied significantly by complication and was highest for pPROM (P<.01)
Conclusions: We describe a large prospectively collected, gestational age-defined, multi-center cohort of deliveries in the extremely low gestational age range. The incidence of preterm labor significantly decreased and the incidence of preeclampsia significantly increased with gestational age among singletons. Compared with singletons, a significantly higher proportion of multiples was delivered for preterm labor and a significantly lower proportion was delivered for preeclampsia. The incidence of antenatal complication varies with advancing gestational age. We suggest there is sufficient gestational age specific heterogeneity to make epidemiological inference sensitive to the parameters of cohort collection – an underappreciated characteristic of low gestational age birth research.

834

Effects of Perinatal Exposure to Heavy Metals and Persistent Organic Pollutants on Neurobehavioral Development in Japanese Children: PCBs Exposure and Neonatal Neurobehavioral Status. Kunihiro Okamura,[*1] Kunihiko Nakai,[2] Keita Suzuki,[2,3,4] Toru Hosokawa,[3] Takeo Sakai.[5] *[1]Department of Obstetrics, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [3]Department of Human Development and Disabilities, Tohoku University Graduate School of Education, Sendai, Miyagi, Japan; [4]Research Fellow of the Japan Society for the Promotion of Science, Tokyo, Japan; [5]Miyagi Children's Hospital, Sendai, Miyagi, Japan.*
Objective: We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to MeHg, PCBs, and dioxins on neurobehavioral development. We report the preliminary results on the association of neonatal behavioral assessment scale (NBAS) with PCB in cord blood and maternal fish intake. Methods: We registered 599 mother-infant pairs. NBAS was performed when the children were three days after birth. Infants were all singleton and full-term (36-42 weeks) gestation. All 209 PCB congeners were measured using GC/MS from the whole cord blood. Maternal fish intake, maternal hair mercury, socioeconomic stats were also analyzed. Results and Discussion: We hypothesized negative associations between total PCBs in cord blood and NBAS. In addition, because fish is rich in nutrients such as PUFA essential for brain development, we also hypothesized a beneficial effect of maternal fish intake. Although we did not yet complete the PCBs assay, the results (n=163) were not in line with our hypotheses. Further measurements of PCBs will reveal the associations of neurobehavioral development with perinatal PCBs exposure and fish intake. (This abstract has previously been presented in part at the Dioxin2006 meeting, Oslo, 21–25 August 2006.)